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DRINKING WATER CRITERIA DOCUMENT FOR ETHYLENE DIBROMIDE (EDB)

Prepared for

OFFICE OF DRINKING WATER

Prepared by

Environmental Criteria and
Assessment Office
Cincinnati OH 45268

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The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on ethylene dibromide. This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of ethylene dibromide
- Toxicokinetics and human exposure to ethylene dibromide
- Health Effects of ethylene dibromide in humans and animals
- Mechanisms of toxicity of ethylene dibromide
- Quantification of toxicological effects of ethylene dibromide

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DOCUMENT DEVELOPMENT

Michael L. Dourson, Ph.D., Document Manager
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Helen H. Ball, M.S., Project Officer
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Authors

Stephen J. Bosch, B.S.
D. Anthony Gray, Ph.D.
Michael W. Neal, Ph.D.
Joseph Santodonato, Ph.D.
Syracuse Research Corporation
Syracuse, NY
(EPA Contract #68-03-3112)

Scientific Reviewers

Randy J.F. Bruins, M.S.
Environmental Criteria and
Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Penelope A. Fenner-Crisp, Ph.D.
Office of Drinking Water
U.S. Environmental Protection Agency
Washington, DC

James W. Holder, Ph.D.
Carcinogen Assessment Group
U.S. Environmental Protection Agency
Washington, DC

Ernest R. Jackson, M.S.
Reproductive Effects Assessment Group
U.S. Environmental Protection Agency
Washington, DC

Editorial Reviewers

Erma Durden, B.S.
Environmental Criteria and
Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Judith Olsen, B.A.
Environmental Criteria and
Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Document Preparation

Technical Support Services Staff: C. Cooper, P. Daunt, C. Fessler, K. Mann, B. Zwayer, Environmental Criteria and Assessment Office, Cincinnati

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LIST OF ABBREVIATIONS

AADI	Adjusted acceptable daily intake
ADI	Acceptable daily intake
AP	Alkaline phosphatase
BUN	Blood urea nitrogen
bw	Body weight
CNS	Central nervous system
DDC	Diethyldithiocarbamate
DNA	Deoxyribonucleic acid
GLC	Gas liquid chromatography
GI	Gastrointestinal
GPT	Glutamic pyruvic tranaminase
GSH	Glutathione
HA	Health advisory
i.p.	Intraperitoneal
LD_{50}	Dose lethal to 50% of recipients
LOAEL	Lowest-observed-adverse-effect level
NOAEL	No-observed-adverse-effect level
PMA	Phorbal myristate acetate
RBC	Red blood count
RNA	Ribonucleic acid
s.c.	Subcutaneous
SCE	Sister-chromatid exchange
SDH	Sorbital dehydrogenase
TWA	Time-weighted average
WBC	White blood count

I. SUMMARY

EDB is a colorless, clear liquid that has been used as a fumigant for soil, grains and fruit, but primarily as a lead scavenger in gasoline and as a general solvent. Based on an EXAMS model, volatilization and biodegradation are the dominant removal processes from ambient waters; volatilization has a half-life of 2-16 days. Chemical and photochemical processes do not appear to proceed at a significant rate when compared with volatilization based on a comparison with similar compounds. Biodegradation appears to take place at a slow but significant rate.

EDB has a high degree of acute toxicity when administered to animals orally or by inhalation. Single dose oral LD_{50}s for rats, rabbits and guinea pigs are in the range of 50-150 mg/kg bw, and death generally results after several hours of exposure to vapor concentrations of $\geq$100 ppm. Hepatotoxicity (i.e., centrilobular necrosis) has been the primary effect investigated in acute oral studies, but inhalation studies with multiple species of rodents indicate that systemic toxicity is widespread; effects typically are described in the lungs, kidneys and spleen (i.e., congestion and edema) as well as the liver. Central nervous system effects (i.e., depression) are pronounced at high vapor concentrations, and deaths due to acute inhalation exposure usually are from respiratory and cardiac failure. Acute dermal studies with rats and rabbits have shown that EDB can be absorbed through the intact skin in amounts sufficient to elicit local and systemic toxic effects and death.

Administration of EDB to rats and mice by gavage at average doses of ~28-77 mg/kg/day for 49-61 weeks produce non-neoplastic and neoplastic lesions in the forestomach (hyperkeratosis, acanthosis and squamous cell carcinoma), liver (degenerative/inflammatory alterations and hepatocellular carcinoma in rats), adrenal cortex (degeneration in rats), circulatory system (hemangiosarcomas in rats) and testes (atrophy in rats and mice). Subchronic inhalation exposure to EDB at concentrations of 25 ppm produces mortality in rats and guinea pigs because of a chemically-induced respiratory infection with no other evidence of gross or microscopic pathology. Chronic inhalation of EDB at the same approximate concentration (20-40 ppm) produces mortality in rats and mice with non-neoplastic and neoplastic effects in the respiratory system (inflammation, hyperplasia/metaplasia and adenoma/carcinoma/adenocarcinoma of the nasal cavity, trachea and/or lungs), circulatory system (hemangiosarcomas), mammary gland (adenomas and carcinomas), testes (atrophy) and other sites (tunica vaginalis, pituitary and adrenal cortex in rats, subcutaneous tissue in mice). Lifetime application of EDB to the skin of mice induces skin and lung papillomas, but skin initiation with EDB followed by promotion with phorbol myristate acetate does not induce tumors at these sites.

Absorption of EDB into the systemic circulation of mammals appears to occur readily as a result of ingestion, inhalation or direct dermal contact. Once absorbed, EDB becomes widely circulated to all tissues. Elimination of EDB from the body is rapid, with urinary excretion of mercapturic acid derivatives (the major derivative being N-acetyl-S-2-hydroxyethyl-L-cysteine) acting as the major pathway of elimination. In mammalian systems, EDB is metabolized by oxidation with glutathione, and alternatively by direct conjugation with glutathione leading to the formation of mercapturic acid derivatives. Both the oxidative and direct conjugative pathways of EDB metabolism are associated with the formation of reactive electrophilic intermediates which are believed to be responsible for the observed toxic effects resulting from exposure. The oxidative pathway appears to be associated with the production of acute tissue damage, whereas the conjugative pathway is more closely linked to DNA binding and, hence, mutagenesis and carcinogenesis.

The mutagenicity of EDB has been demonstrated _in vitro_ in bacteria. In these assays, there is conflicting evidence as to whether EDB was a direct acting mutagen, is inactivated by the mammalian metabolic activation system (S-9) or required metabolic activation to form the ultimate mutagen. The difficulty in assessing the mutagenicity of volatile compounds in the standard assay system as a result of loss may have accounted for the variations observed in results. Increases in mutations have also been demonstrated in eukaryotic systems employing fungi, cultured mammalian cells and fruit flies. In the dominant lethal assay, the only mammalian test system used, EDB, failed to elicit a positive response. A single mutagenicity assay with _Salmonella typhimurium_ as the test organism indicates the possibility that some of the mutagenic activity of EDB may result from a high molecular weight contaminant; however, some activity still remained with freshly distilled EDB. In addition, there is limited evidence that EDB may cause SCEs, chromosomal aberrations or polyploidy.

A number of studies demonstrate both directly and indirectly that EDB can interact with DNA. EDB preferentially kills bacteria deficient in DNA repair capability as compared with competent cells, increases unscheduled DNA synthesis in cultured cells and increases single strand breaks in DNA in both cultured cells and whole animals. Direct evidence for interactions with DNA is provided by binding of radiolabeled EDB to DNA in both in vitro and in vivo test systems. The major EDB DNA adduct is identified tentatively as S-[2- (N^7-guanyl)ethyl] GSH.

The teratogenic potential of EDB has been investigated only in rats and mice. Nearly continuous vapor exposure (23 hours/day) to 20 ppm EDB during organogenesis produces reduced maternal feed consumption and some fetal ossification anomalies in both species. Similar exposure to higher concentrations produces dose-related maternal and fetotoxicity, more pronounced fetal skeletal anomalies and limited evidence of fetal soft-tissue effects (i.e., hydrocephaly in rats at 32 ppm, but not at 80 ppm). The skeletal anomalies are not dose-related and appear to be a reflection of delayed development and maternal toxicity rather than teratogenesis. Intraperitoneal injection of EDB during pregnancy at a dose that produces organ weight changes in maternal rats does not produce fetotoxicity or fetal developmental abnormalities.

The antispermatogenic effects of EDB were recognized nearly 25 years ago when it was reported that bulls exposed orally to a dose of 2 mg/kg/day in the diet developed transient sperm abnormalities in as little as 2 weeks. The spermicidal effect of EDB can be demonstrated in bulls in the absence of any other obvious signs of systemic toxicity or behavioral alteration. Similar reversible antispermatogenic effects with EDB can be produced in rams, although the doses required may be somewhat higher than for the bull. Rats appear to be relatively resistant to the spermicidal effects of EDB.

Epidemiologic evidence concerning the adverse effects of EDB exposed human populations is quite limited. Historical case reports and controlled clinical studies have established that EDB is a severe skin irritant that can be absorbed into the circulation from dermal contact. The lethal oral dose in humans for a single exposure to EDB can be as low as 65 mg/kg. Investigations of relatively small groups of workers potentially exposed to EDB reveal no firm evidence of increased cancer risk, antispermatogenic effects or death from other target organ damage. Weak evidence exists that associates reduced fertility in males with occupational exposure to EDB. It must be stressed, however, that the available epidemiologic data are inadequate to allow for identification of adverse effects that occur with relatively low frequency or that result from long-term low-level exposures.

Because of the documented antispermatic effect of EDB in animals, human males should be regarded as a population at special risk. An additional risk to EDB exposure involves situations where humans may be exposed simultaneously to inhibitors of drug metabolizing enzymes (i.e., therapeutic use of disulfiram in the medical management of alcoholism).

1-Day and 10-day HAs were derived for EDB from data show an antispermatic effect of EDB in rams at daily doses below the available rat and mouse data. The reproductive effect in rams is considered relevant to humans; thus derivation of 1- and 10-day HAs yield values for both advisories of 8 or 38 μg/ℓ for a 10 kg child or a 70 kg adult, respectively. A lifetime AADI was not calculated because appropriate toxicity data were not available for this after chronic exposure. The U.S. EPA Carcinogen Assessment Group estimated lifetime cancer risks for EDB from the NCI (1978) tumor response study. These values are given in Table VIII-1 for a risk of 10^{-4}.

II. PHYSICAL AND CHEMICAL PROPERTIES

Synonyms: Ethylene dibromide
1,2-Dibromoethane
Ethylene bromide
EDB
Glycoldibromide

Structure: $BrCH_2CH_2Br$

Molecular Formula: $C_2H_4Br_2$

Molecular Weight: 187.88

CAS Registry Number: 106-93-4

RTECS Number: KH92750000

Physical and Chemical Properties

EDB is a colorless, clear liquid that has been used as a fumigant for soil, grains and fruit, but primarily as a lead scavenger in gasoline and as a general solvent (Verschueren, 1983). The physical properties of EDB are listed in Table II-1.

Stability in Water

Based on an EXAMS model as well as experimental results, the dominant removal processes for EDB from ambient surface waters are volatilization and biodegradation. Also based on an EXAMS model using input parameters from Verschueren (1983) and Stenger (1978), the volatilization half-life will range from 2-16 days, depending on the environment. Experimental results support the rapid volatilization of EDB (Chiou et al., 1980; Mackay and Yeun, 1983; Neely, 1976).

TABLE II-1

Physical Properties of EDB[a]

Molecular weight	187.87 (Weast, 1980)
Boiling point	131.4°C
Melting point	9.9°C
Density	2.1792 g/mℓ
Vapor pressure	11 torr (20°C) (Verschueren, 1983)
Octanol/water partition coefficient	135[b]
Water solubility	4,310 mg/ℓ (Verschueren, 1983)
Lipid solubility	soluble in alcohol, ether, benzene, acetone (Weast, 1980)
Conversion factors	1 mg/m³ = 0.13 ppm 1 ppm = 7.81 mg/m³

[a] Source: Stenger, 1978, except where otherwise noted

[b] Back calculated from the solubility (Lyman, 1982)

Based on data derived for similar chloroethanes, chemical and photochemical removal processes do not appear to be significant in the environment (Callahan et al., 1979). Ehrenberg et al. (1974) reported the hydrolysis rate constant for EDB in distilled, CO_2-free water at 20°C to be 6×10^{-6} hr^{-1}, which leads to a half-life of ~13 years. This value is close to the 14-year half-life reported in the RPAR for EDB (42 FR 63139). Nonetheless, distilled, CO_2-free water is not representative of the environment; a somewhat faster rate might be expected in the environment.

In sharp contrast to these are the half-lives of 5-10 days, reported in at least two documents (Johns, 1976; Brown et al., 1975), and 5000 years estimated by Radding et al. (1977). The half-life of 5-10 days appears to based on the work of Oliver and Weber (1934), who report the hydrolysis of EDB in a water acetone mixture at elevated temperatures. This solvent system most likely would not yield environmentally significant values. The half-life values of 13-14 years, consequently, appear to be the most environmentally reasonable half-lives.

Only one report on the biodegradation of EDB was found in the available literature. Castro and Belser (1968) reported that a soil inoculum added to an autoclaved soil with added growth medium resulted in a 97% reduction in EDB concentration in 8 weeks. Ethylene and bromide were reported as products. Based on data for similar compounds, EDB would be expected to biodegrade slowly in the environment (Callahan et al., 1979).

Summary

Based on an EXAMS model, volatilization is the dominant removal process from ambient surface waters with a half-life of 2-16 days. Chemical and photochemical processes do not appear to proceed at a significant rate when compared with volatilization (based on a comparison with similar compounds (Callahan et al., 1979). Biodegradation appears to take place at a slow but significant rate (Castro and Belser, 1968).

III. TOXICOKINETICS

An understanding of the bioavailability and metabolic fate of EDB is essential to the elucidation of its mechanism of toxic action. Several lines of investigation have been pursued with EDB that involved determination of absorbed dose and tissue localization, characterization of chemical metabolites and adducts covalently bound to macromolecules (protein, DNA, RNA) and studies of biochemical reactions involved in activation and detoxication.

Absorption

Information regarding the bioavailability of EDB can be obtained from numerous tissue distribution and metabolism studies conducted with experimental mammals. The available data indicate that uptake of EDB readily occurs in rats following exposure by inhalation (Watanabe et al., 1978), oral intubation (van Bladeren et al., 1980; Plotnick et al., 1979; Nachtomi, 1981; Batti et al., 1982; Jones and Edwards, 1968) and i.p. injection (Hill et al., 1978). Significant absorption of EDB also occurs in guinea pigs exposed by dermal application (Jakobson et al., 1982) and by i.p. injection (Plotnick and Conner, 1976).

Quantitative data on the extent of uptake by various routes of absorption generally are not available for EDB, since no studies were designed to yield this information. It may be inferred, however, that uptake from the GI tract of rats is extensive, since urinary excretion accounts for up to 72% of orally administered ^{14}C-EDB in the rat (Plotnick et al., 1979; van Bladeren et al., 1980), with fecal excretion accounting for ~2% (Plotnick et al., 1979).

Jakobson et al. (1982) published the only available data on the uptake of EDB into the blood. Guinea pigs of both sexes were exposed to 1 mℓ of EDB contained within a glass ring held in direct contact with a clipped area of skin on the back. Blood was collected through a catheter in the carotid artery at timed intervals for up to 6 hours of exposure. The test substance was not depleted from the glass ring depot during the exposure period. The concentration of EDB in the blood showed a marked initial increase during the first 0.5 hour, reaching a maximum level of ~2.1 μg/mℓ at 1 hour (measured by head space gas chromatographic analysis). The blood concentration reached a plateau after 1 hour and dropped to ~1.8 μg/mℓ after 6 hours when the experiment was terminated. Among the 10 organic chemicals evaluated in this study (benzene, carbon tetrachloride, 1,2-dichloroethane, EDB, n-hexane, 1,1,2,2-tetrachloroethane, tetrachloroethylene, toluene, 1,1,1-trichloroethane, trichloroethylene), only 1,1,1-trichloroethane achieved higher peak blood levels (~2.5 μg/mℓ) than EDB.

Distribution

The tissue distribution of ^{14}C-EDB has been studied following its oral administration to rats and i.p. injection in guinea pigs and mice. The results of all studies are in general agreement. Plotnick et al. (1979) treated male Sprague-Dawley rats with a single oral dose of uniformly radiolabeled [U-^{14}C]-EDB (15 mg/kg) in corn oil and placed the animals in metabolism cages. One group of six rats was sacrificed 24 hours after treatment and a second group of six rats sacrificed after 48 hours. Tissue analyses of ^{14}C activity revealed the highest concentrations to be in the liver, kidneys and spleen (Table III-1). The authors were not able to account for all of the administered radioactivity, although loss of EDB

TABLE III-1

Distribution of ^{14}C in Selected Tissues and Body Fluids of Male Rats 24 and 48 Hours After a Single Oral Dose of 15 mg/kg [U-^{14}C]-EDB[a]

Tissue	Tissue Concentrations[b] 24 Hours	Tissue Concentrations[b] 48 Hours	Percent of Dose[c] 24 Hours	Percent of Dose[c] 48 Hours
Liver	4.78 ± 0.24	2.87 ± 0.33	1.79 ± 0.07	1.10 ± 0.12
Kidneys	3.32 ± 0.42	1.06 ± 0.16	0.21 ± 0.02	0.08 ± 0.01
Spleen	1.00 ± 0.03	0.66 ± 0.03	0.02 ± <0.01	0.01 ± <0.01
Testes	0.49 ± 0.05	0.19 ± 0.02	0.04 ± <0.01	0.01 ± <0.01
Brain	0.41 ± 0.04	0.17 ± 0.02	0.02 ± <0.01	0.01 ± <0.01
Fat[d]	0.35 ± 0.04	0.44 ± 0.06	0.15 ± 0.02	0.20 ± 0.03
Blood[e]	0.90 ± 0.05	0.64 ± 0.07	0.59 ± 0.03	0.43 ± 0.04
Plasma	0.46 ± 0.04	0.22 ± 0.02	---	---
Urine	---	---	72.38 ± 0.98[f]	73.54 ± 2.80[g]
Feces	---	---	1.65 ± 0.28[f]	2.42 ± 0.54[g]
Total recovery	---	---	76.85	77.8

[a] Source: Adapted from Plotnick et al., 1979

[b] Values represent mean concentrations in µg/g or µg/mℓ (expressed as parent compound) ± S.E.M. of duplicate determinations on six animals.

[c] Values represent the mean percentage of the administered radioactivity ± S.E.M. of duplicate determinations on six animals.

[d] Assumed 6% of body weight

[e] Assumed 9% of body weight

[f] n = 12 (includes 24-hour samples obtained from rats killed 48 hours after compound administration)

[g] Cumulative 48-hour excretion

through the expired air was not measured and thus may partially explain the low recovery.

A tissue distribution study of [U-^{14}C]-EDB in guinea pigs was undertaken by Plotnick and Conner (1976) because of the close similarities in metabolic biotransformation pathways between guinea pigs and humans. Eighteen male Hartley guinea pigs received a single i.p. injection of 30 mg/kg [U-^{14}C]-EDB in corn oil and were placed in metabolism cages for up to 72 hours. Animals were serially sacrificed at 4, 8, 12, 24, 48 and 72 hours following treatment and tissues analyzed for ^{14}C activity. The cumulative urinary and fecal excretion of radioactivity represented 66 and <3% of the dose, respectively, at the end of the 72-hour period. The major target organs for localization of radioactivity in the guinea pig were similar to those observed in the rat, although the percent of dose recovered in guinea pig tissues was generally higher than for the rat (Table III-2). Recovery of administered radioactivity was not complete; however, the authors estimated that ~10-12% of the dose was excreted in the expired air.

A limited study on the tissue distribution of [U-^{14}C]-EDB in mice was reported by Edwards et al. (1970). Male RF/Hiraki mice received 40 mg/kg [U-^{14}C]-EDB by i.p. injection in arachis oil. Animals (number not specified) were sacrificed at 1, 3 and 24 hours after treatment and ^{14}C activity determined in various tissue digests. No indication was given of ^{14}C counting efficiency or total recovery of administered radioactivity. The results indicated a rapid and widespread tissue distribution of ^{14}C, with major localization at 1 and 3 hours in the intestines, kidneys, liver, blood, fat and spleen. Clearance of radioactivity from most tissues was

TABLE III-2

Percent of Administered ^{14}C in Selected Tissues and Body Fluids of Male Guinea Pigs at Various Time Intervals Following Intraperitoneal Administration of 30 mg/kg of ^{14}C-EDB[a]

Organ	4 hour	8 hour	12 hour	24 hour	48 hour	72 hour
Liver	16.29 ± 2.42[b]	13.65 ± 0.39	10.50 ± 2.13	4.72 ± 0.21	2.12 ± 0.07	2.16 ± 0.21
Kidneys	6.00 ± 0.42	5.69 ± 0.43	3.31 ± 0.17	1.64 ± 0.45	0.31 ± 0.01	0.24 ± 0.02
Stomach[c]	1.14 ± 0.44	0.52 ± 0.20	0.62 ± 0.08	0.38 ± 0.07	0.18 ± 0.02	0.18 ± 0.04
Lungs	0.35 ± 0.06	0.38 ± 0.09	0.37 ± 0.01	0.24 ± 0.01	0.12 ± 0.01	0.10 ± 0.01
Pancreas	0.31 ± 0.10	0.36 ± 0.06	0.33 ± 0.02	0.20 ± 0.03	0.07 ± 0.01	0.06 ± 0.01
Testes	0.16 ± 0.04	0.17 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Heart	0.13 ± 0.02	0.16 ± 0.02	0.12 ± 0.01	0.10 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
Brain	0.12 ± 0.02	0.16 ± 0.02	0.14 ± 0.01	0.13 ± 0.01	0.07 ± 0.01	0.05 ± 0.00
Adrenals	0.09 ± 0.02	0.10 ± 0.04	0.04 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Spleen	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.02	0.03 ± 0.00	0.02 ± 0.01
Urine[d]	14.9 ± 1.0	26.3 ± 10.1	43.3 ± 8.1	46.0 ± 4.8	54.3 ± 3.4	65.9 ± 4.6

[a] Source: Adapted from Plotnick and Conner, 1976

[b] Values represent the Mean ± S.E.M. of duplicate determinations on three animals at each time interval.

[c] Including stomach contents.

[d] Cumulative excretion.

nearly complete after 24 hours, with the exception of kidneys and stomach where 1% of the administered dose/g wet tissue remained in each. In addition, whole blood and plasma retained 6.2 and 2.6%, respectively, of the administered dose/g wet tissue after 24 hours. These results generally show less retention of radioactivity in mice than with rats and guinea pigs, although ^{14}C levels in the blood and plasma of mice seem inexplicably elevated.

Bioaccumulation/Retention

Tissue distribution studies with [U-^{14}C]-EDB administered to rats (Plotnick et al., 1979) and guinea pigs (Plotnick and Conner, 1976) indicate little potential for bioaccumulation. Under special conditions, however, the tissue retention of EDB can be increased by concurrent exposure to certain modifiers of enzyme activity.

Plotnick et al. (1979) exposed male Sprague-Dawley rats to disulfiram, an inhibitor of aldehyde dehydrogenase, at a concentration of 0.05% in the diet for a period of 12 days. After fasting overnight, the rats were given a single oral dose of 15 mg/kg [U-^{14}C]-EDB in corn oil. The tissue distribution of ^{14}C activity was measured in disulfiram-pretreated rats at 24 and 48 hours, and the results compared with those from rats fed a control diet before administration of the test substance. At both 24 and 48 hours following administration of [U-^{14}C]-EDB, tissue concentrations of ^{14}C activity were significantly higher ($p<0.05$) in the liver, kidneys, spleen, testes and brain of rats receiving disulfiram in the diet. These observations also were associated with a significantly depressed urinary excretion of radioactivity in the first 24 hours and a reduced rate of clearance of ^{14}C from liver, kidneys, spleen, testes and brain of disulfiram-treated rats. In addition, an increased uptake of ^{14}C into liver nuclei was demonstrated in the disulfiram group. Increased tissue levels of ^{14}C activity in the disulfiram group correlated with target organs affected by EDB in chronic toxicity bioassays (e.g., tumors of the liver, spleen and kidney, and atrophy of the testes).

Retention of EDB in mammalian tissues, although quite limited (see Distribution), may nevertheless be toxicologically significant. In particular, irreversible binding to macromolecules is likely to be a necessary prerequisite to many toxic and carcinogenic effects. Hill et al. (1978) evaluated macromolecular binding in six rats given i.p. injections of ~3 mg/kg [U-^{14}C]-EDB (30.4 μCi) in 0.2 mℓ ethanol and sacrificed 24 hours later. Protein, DNA and RNA were isolated from various tissues and analyzed for irreversibly bound ^{14}C activity. The highest concentrations of bound radioactivity were found in the liver, kidneys, lungs and small intestine (Table III-3). The binding reaction was studied further _in vitro_ using microsomes prepared from rat livers. Both enzyme-mediated and nonenzymatic binding of [U-^{14}C]-EDB to microsomes was observed. The enzyme-mediated binding reaction was enhanced by pretreatment of rats with phenobarbital but not with benz[a]anthracene, thus implying that cytochrome P-450 and not cytochrome P-448 was involved.

Banerjee and Van Duuren (1979) also studied the enzymatic and nonenzymatic binding of [U-^{14}C]-EDB to mouse liver and forestomach microsomal protein _in vitro_. Using native microsomes isolated from B6C3F$_1$ mice,

TABLE III-3

Irreversible Binding of Radioactivity from [U-^{14}C]-EDB to Protein, RNA and DNA of Tissues of Rats*

Tissue	Protein	DNA	RNA
Brain	69	5	54
Heart	39	7	34
Lung	120	12	81
Liver	240	63	300
Spleen	70	26	140
Kidney	260	66	640
Testis	78	10	110
Stomach	63	15	69
Large intestine	66	16	98
Small intestine	140	22	160
Muscle	10	4	28

*Modified from Hill et al., 1978. Rats were given i.p. injection of test substance (30.4 μCi; 4.2 μmol) and sacrificed 24 hours later. Results are the averages of two separate experiments employing three rats each.

binding of radioactivity to liver microsomal protein was ~20 times greater than to forestomach microsomal protein. Binding was reduced by 96 and 99% in stomach and liver, respectively, when denatured microsomes were substituted for native microsomes. Likewise SKF-525A, an inhibitor of the microsomal metabolism of various substances, significantly reduced the binding of [U-^{14}C]-EDB, thus indicating the importance of metabolic activation as an enhancer of macromolecular binding.

The enzymatic binding of EDB to microsomal protein was confirmed in a study by Nachtomi (1981) using rat liver microsomal preparations. In agreement with previous investigators, Nachtomi (1981) showed that phenobarbital pretreatment of rats enhanced the binding reaction, and diethyldithiocarbamate, a metabolic inhibitor, interfered with protein binding.

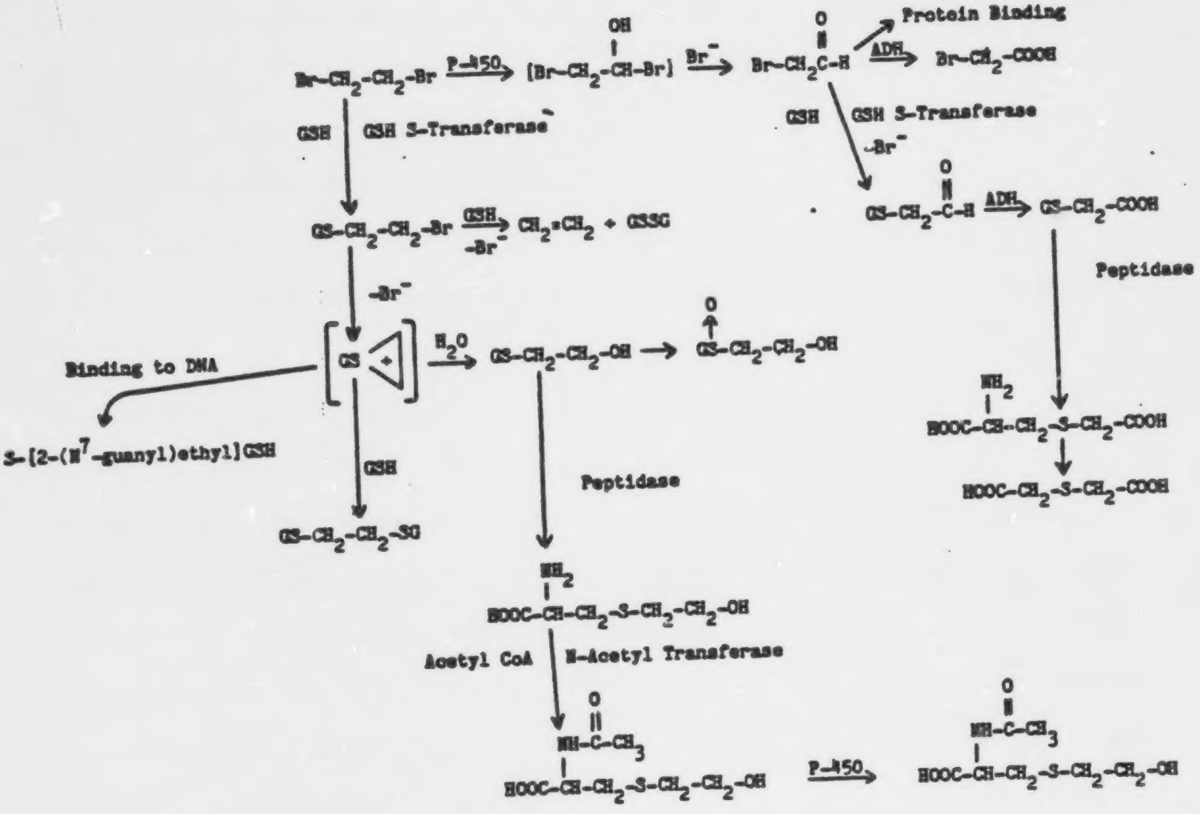
Metabolism

The pathway of biotransformation for EDB is believed to be the controlling factor for its biologic activity. Since the early studies by Heppel and Porterfield (1948) demonstrating that rat liver cytosol fractions enzymatically dehalogenated EDB and other halogenated aliphatic hydrocarbons, numerous analytical and mechanistic studies were undertaken beginning in the 1960s.

When administered to rats, EDB was found to be converted to mercapturic acid derivatives that appear in the urine (Nachtomi et al., 1966; Nachtomi, 1970). The principal mercapturic acid derivative is N-acetyl-S-2-hydroxyethyl-L-cysteine, although other related metabolites are formed, all of which are derived from glutathione adducts (Jones and Edwards, 1968; van

Bladeren et al., 1980, 1981a). Surprisingly, no bromine containing glutathione conjugates were found. The fact that 1,2-dibromoethane forms glutathione conjugates is not surprising since it is known that most alkyl halides are sufficiently electrophilic and lipophilic to be good substrates for glutathione conjugation (Chasseaud, 1979). It is important to note that many of the glutathione conjugation reactions catalyzed by the glutathione-S-transferases also proceed rapidly in the absence of enzyme catalysis. However, in the case of EDB, the presence of both glutathione and glutathione-S-transferase are required for the formation of conjugates (Nachtomi, 1970) and for *in vitro* covalent binding to DNA from rat liver or calf thymus (Ozawa and Guengerich, 1983). Moreover, the presence of glutathione is thought to be required for expression of mutagenicity by EDB (van Bladeren et al., 1980; Rannug, 1980), which is paradoxical in view of the well-established role of glutathione conjugation as a detoxication mechanism.

Recent studies with rats have provided strong evidence that two pathways of metabolic bioactivation exist for EDB, each producing a reactive metabolite capable of eliciting toxic effects (Figure III-1). Metabolism by the mixed function oxidase system produces a putative gemhalohydrin, which dehydrobrominates to form 2-bromoacetaldehyde (Hill et al., 1978; Shih and Hill, 1981). 2-Bromoacetaldehyde could subsequently react with glutathione, become bound to protein thiols or become converted to the corresponding acid by the action of aldehyde dehydrogenase. EDB also can react directly with glutathione by the action of glutathione-S-transferase to form S-(2-bromoethyl)glutathione (Livesey and Anders, 1979; Anders and Livesey, 1980; Livesey et al., 1982). This derivative can be attacked subsequently by a second molecule of glutathione at the sulfur atom of the conjugate to yield


Br-CH2-CH2-Br
P-450
[Br-CH2-CH-Br]
OH
Br⁻
Br-CH2C-H
O
Protein Binding
ADH
Br-CH2-COOH
GSH
GSH S-Transferase
-Br⁻
GS-CH2-C-H
ADH
GS-CH2-COOH
Peptidase
NH2
HOOC-CH-CH2-S-CH2-COOH
HOOC-CH2-S-CH2-COOH
GSH
GSH S-Transferase
GS-CH2-CH2-Br
GSH
-Br⁻
CH2=CH2 + GSSG
-Br⁻
GS
+
H2O
GS-CH2-CH2-OH
GS-CH2-CH2-OH
O
Binding to DNA
S-[2-(N7-guanyl)ethyl]GSH
GSH
GS-CH2-CH2-SG
Peptidase
NH2
HOOC-CH-CH2-S-CH2-CH2-OH
Acetyl CoA
N-Acetyl Transferase
NH-C-CH3
O
HOOC-CH-CH2-S-CH2-CH2-OH
P-450
NH-C-CH3
O
HOOC-CH-CH2-S-CH2-CH2-OH

P-450 = cytochrome P-450
GSH = glutathione, reduced
ADH = aldehyde dehydrogenase

GS =

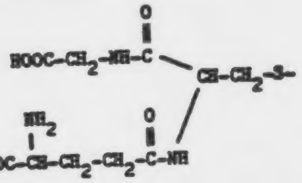

HOOC-CH2-NH-C
O
CH-CH2-S-
NH2
O
HOOC-CH-CH2-CH2-C-NH

FIGURE III-1

Proposed Metabolism of EDB
Source: Anders and Livesey, 1980

glutathione disulfide, ethylene and bromide ion, which are considered to be detoxication products. Alternatively, S-(2-bromoethyl)glutathione may be converted by a putative ethylene-S-glutathionyl-episulfonium ion, a highly reactive alkylating agent, to S,S'-ethylene-bis(glutathione) or S-(2-hydroxyethyl)glutathione. The episulfonium ion intermediate is believed to be the species responsible for binding to DNA and hence mutagenesis.

Both the oxidative and conjugative pathways of EDB metabolism appear to be operative in rats (van Bladeren et al., 1980, 1981). Studies on the *in vivo* metabolism of tetradeutero-EDB in the rat revealed that the oxidative pathway predominates over the conjugative pathway by a 4:1 ratio (van Bladeren et al., 1981). The available evidence suggests that the reactive metabolite produced by the oxidative pathway, 2-bromoacetaldehyde, is important in protein binding and associated histopathological changes such as liver damage (Shih and Hill, 1981; Nachtomi, 1981). The conjugative pathway, on the other hand, is more closely associated with DNA binding and mutagenesis (Hill et al., 1978; van Bladeren et al., 1980, 1981a; Sundheimer et al., 1982).

Ozawa and Guengerich (1983) recently reported the structure of the major DNA adduct formed with EDB in the presence of glutathione and glutathione-S-transferase as S-[2-(N^7-guanyl)ethyl]glutathione. Studies with isolated rat hepatocytes indicated that a high percentage of [U-^{14}C] EDB metabolites become irreversibly bound to nucleic acids, apparently by attack of the N^7-guanyl on S-(2-bromoethyl)glutathione. The stoichiometric binding of glutathione and EDB to DNA supported the hypothesis of an intermediate episulfonium ion.

Excretion

In the rat, orally administered EDB is excreted primarily in the urine as mercapturic acid derivatives (Nachtomi et al., 1966; Jones and Edwards, 1968; Nachtomi, 1970; Plotnick et al., 1979). Unchanged EDB apparently is not excreted in the urine, although it may be eliminated in small percentages in the expired air. Fecal excretion is not a significant pathway of elimination for orally administered 1,2-dibromoethane. Patterns of elimination by the guinea pig are similar to the rat (Plotnick and Conner, 1976).

Elimination of inhaled EDB by the rat follows the same pattern as with oral exposures. Watanabe et al. (1978) studied the elimination of ^{14}C-labelled EDB in rats following 6-hour inhalation exposures to a concentration of 7, 25 or 75 ppm in air. At all exposure levels, urinary excretion accounted for ~80% of the recovered radioactivity during the 48-hour period following exposure. Rates of urinary excretion of radioactivity indicated a half-life for elimination in the range of 5.1-5.6 hours.

Summary

Exposure to EDB by ingestion (rats), i.p. injection (guinea pigs), inhalation (rats) or dermal contact (guinea pigs) results in rapid absorption (Hill et al., 1978; van Bladeren et al., 1980; Plotnick et al., 1979; Watanabe et al., 1978). Intestinal absorption of ingested doses of EDB is essentially complete (Plotnick et al., 1979; van Bladeren et al., 1980). Once absorbed, EDB is distributed widely to the tissues, with the primary sites of localization (liver, kidneys) corresponding to the major target organs for toxic effects (Plotnick et al., 1979; Plotnick and Conner, 1976). 1,2-Dibromoethane shows little, if any, tendency for bioaccumulation in mammalian systems, although covalent binding to cell macromolecules occurs, which may be toxicologically significant.

The metabolism of EDB has been studied thoroughly in the rat, both _in vivo_ and _in vitro_ using subcellular liver preparations (Nachtomi, 1970; Nachtomi et al., 1966; van Bladeren et al., 1980, 1981a). A convincing body of evidence indicates that separate oxidative and conjugative pathways of metabolism exist for EDB, with the oxidative pathway predominating under normal conditions (van Bladeren et al., 1981; Shih and Hill, 1981). Ultimately identical mercapturic acids are excreted in the urine, the major urinary metabolite being N-acetyl-S-2-hydroxyethyl-L-cysteine. Both the oxidative and conjugative routes of metabolism involve a reactive, and therefore potentially toxic, intermediate. Microsomal oxidation leads to bromoacetaldehyde formation which is capable of binding to protein thiols and thus producing tissue damage. Conjugation of glutathione with EDB produces S-(2-bromoethyl)glutathione that can rearranged to a putative ethylene-S-glutathionyl-episulfonium ion, which is capable of binding to DNA (Livesey and Anders, 1979; Livesey et al., 1982). Involvement of an episulfonium ion in the metabolism of EDB would explain the paradoxical role of glutathione conjugation in the mutagenic bioactivation of EDB. An adduct has been isolated (S-[2-(N^7-guanyl)ethyl]-glutathione) in which glutathione in the presence of EDB becomes bound to DNA through an ethylene bridge (Ozawa and Guengerich, 1983); the ethylene bridge comes from the two carbon atoms in the EDB molecule. This finding supports the hypothesis that S-(2-bromoethyl)-glutathione or the resulting episulfonium ion is responsible for the observed mutagenic and likely the carcinogenic effects of EDB.

IV. HUMAN EXPOSURE

Humans may be exposed to chemicals such as EDB from a variety of sources, including drinking water, food, ambient air, occupational settings and consumer products. This analysis of human exposure to EDB is limited to drinking water, food and ambient air because those media are considered to be sources common to all individuals. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and on several factors over which there is little control. Where one lives, works and travels, what one eats, and physiologic characteristics related to age, sex and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

Detailed information concerning the occurrence of and exposure to EDB in the environment is presented in another document entitled "Occurrence of Pesticides in Drinking Water, Food and Air" (Johnston et al., 1984). This chapter summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food and air.

In the Exposure Estimation section of this chapter, available information is presented on the range of human exposure and intake for EDB from drinking water, food and ambient air for the 70 kg adult male. It is not possible to provide an estimate of the number of individuals experiencing specific combined exposures from those three sources. However, the Summary section of this chapter provides some insight into the range of intake values suggested by the available data.

Exposure Estimation

Drinking Water. Levels of EDB in drinking water vary from one location to another. Although the data on levels of EDB in drinking water are limited, reported levels ranged from "undetectable" to 560 µg/ℓ (JRB Associates, 1981). Sufficient monitoring data are not available to determine regional variations in levels of exposure to EDB.

The daily intake of EDB from drinking water was estimated using a range of 0.0-560 µg/ℓ EDB in drinking water. Assuming that a 70 kg adult male consumes 2 ℓ of water/day, estimated intake of EDB would range between 0.0 and 16 µg/kg/day. The values presented, however, do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

Diet. The dietary intake of EDB in the United States was estimated by EPA in Position Document 2/3 and Position Document 4 and in two technical support documents (U.S. EPA, 1983, 1984a,b). Dietary intakes were calculated for the following uses of EDB: soil fumigation, post-harvest fumigation of grain, fumigation of grain milling machinery, and quarantine fumigation of citrus and tropical fruits. These estimated dietary intakes are summarized in Table IV-1.

TABLE IV-1

Estimated Dietary Intakes of EDB by the General U.S. Population[a]

Use	Intake Estimates for the U.S. Population (µg/kg/day), as presented in:			
	Position Document 2/3		Position Document 4	Technical Support Documents
	Average	Maximum		
Soil fumigation	0.000259	0.0041	--	--
Grain fumigation[b]	0.00078	0.041	0.09 (maximum)	0.0055 0.0063[c] 0.0128[d]
Quarantine fumigation				
Citrus	0.0105	0.035	0.00041 (non-CA) 0.0069 (CA)	0.00048 0.00034[c] 0.00094[d]
Tropical fruits	0.0017	0.0041	0.00425	

[a] Sources: U.S. EPA, 1983, 1984a,b

[b] Post-harvest and milling

[c] 13- to 19-year-old male

[d] 1- to 6-year-old child

In Position Document 2/3, EPA calculated the dietary intake for the United States population resulting from EDB fumigation of citrus and tropical fruit. EDB residue levels were multiplied by the percent of crop treated and the percent of the human diet that the commodity represents. The average dietary intake of EDB from citrus was estimated to be 0.0105 μg/kg/day; the maximum intake was estimated to be 0.035 μg/kg/day (U.S. EPA, 1983). In Position Document 4, dietary estimates for EDB intake from citrus were reevaluated to account for the contribution from Mexican citrus and to use 1982 estimates of the percentage of fresh fruit treated. In addition, separate dietary burden estimates were calculated for residents of California to account for the higher percentage of fumigated fruit consumed in that state. Dietary intake of EDB from citrus was estimated to be 0.00041 μg/kg/day for non-California citizens and 0.0069 μg/kg/day for California residents. In Position Document 2/3, the average intake of EDB from tropical fruit was estimated to be 0.0017 μg/kg/day. The maximum intake was calculated to be 0.0041 μg/kg/day. A later estimate in Position Document 4, based on an intake of 12 fruits per year and an average EDB residue level of 31 μg/kg, was 0.00425 μg/kg/day for a 60 kg person (U.S. EPA, 1983).

Dietary intakes of EDB from grain and grain milling fumigation and quarantine fumigation uses were estimated in 1984 by EPA in two technical support documents (U.S. EPA, 1984a,b). Individual exposure estimates for each respondent were analyzed by subgroups (i.e., age, sex, ethnic group, pregnant women, nursing women) and by region and season. Based on the monitoring data in the technical support documents for grain and grain milling fumigation (U.S. EPA, 1984a), the mean dietary burden for the U.S. population from consumption of EDB-contaminated grain was estimated to be 0.0055 µg/kg/day. For males between 13 and 19 years old, the mean dietary intake of EDB from grain was estimated to be 0.0063 µg/kg/day. For children between 1 and 6 years old, the average estimated dietary intake of EDB from grain was 0.0128 µg/kg/day (U.S. EPA, 1984a).

Dietary intake of EDB from fresh citrus was determined in the technical support document for quarantine fumigation (U.S. EPA, 1984b). The national average dietary intake of EDB from fresh citrus was estimated to be 0.00048 µg/kg/day. In the 13- to 19-year-old adult male, intake of EDB from citrus was estimated to be 0.00034 µg/kg/day, and for children 1-6 years old, the estimated intake was 0.00094 µg/kg/day (U.S. EPA, 1984b).

Using the data from the latest EPA estimates of intakes from grain and citrus (the major dietary contributors), the daily adult intake of EDB for a 13- to 19-year-old male is estimated to be 0.0066 µg/kg/day.

Dietary levels of EDB may vary somewhat with geographic location (Table IV-2). The highest dietary intakes were estimated to occur in the South, where the combined intake of EDB from citrus and grain totals 0.0062 µg/kg/day.

TABLE IV-2

Estimated Daily Intake of EDB by Geographic Region*

Region	Dietary Intake (μg/kg/day)	
	Citrus	Grain
Northeast	0.00052	0.0051
North Central	0.0005	0.0052
South	0.0003	0.0059
West	0.00074	0.0052

*Sources: U.S. EPA, 1984a,b

Tolerance levels for EDB in and on raw agricultural commodities have recently been revised. On March 25 and April 23, 1984, EPA published notices in the Federal Register (49 FR 17144; 49 FR 22082) to:

(1) Revoke exemptions from tolerances for inorganic and organic bromides resulting from post-harvest use of EDB on barley, corn, oats, popcorn, rice, rye, sorghum (milo) and wheat (40 CFR 180.126; 40 CFR 180.1006);

(2) Establish a tolerance for EDB residues per se of 900 µg/kg on the grains listed above as a post-harvest fumigant (40 CFR 180.397);

(3) Revoke a food additive regulation [21 CFR 193.250(b)] for residues of inorganic bromide in or on certain processed foods;

(4) Revoke tolerances for residues of inorganic bromides in citrus fruit and papayas (40 CFR 180.146); and

(5) Establish tolerances for EDB per se on citrus and papayas of 250 µg/kg (of which no more than 30 µg/kg is present in the edible pulp) (40 CFR 180.397).

Additional tolerances for inorganic and organic bromides resulting from the uses of EDB are presented in Table IV-3.

Air. Levels of EDB in the atmosphere vary from one location to another. The highest level of EDB reported in the atmosphere was 240,000 ng/m³ (240 µg/m³) (Brodzinsky and Singh, 1982). High levels, averaging greater than 5000 ng/m³ (5 µg/m³), have been detected in other areas. Typical levels, however, are somewhat lower. Brodzinsky and Singh (1982) calculated mean air levels of EDB for rural/remote areas, urban/suburban areas and source-dominated areas of 0.0 ng/m³ (0.0 µg/m³), 200 ng/m³ (0.2 µg/m³) and 1500 ng/m³ (1.5 µg/m³), respectively.

TABLE IV-3

Inorganic and Organic Bromide Tolerances Resulting from EDB Use[a]

Commodity	Tolerance (μg/kg)
Soil fumigation uses[b]	
Broccoli	75,000
Carrots	75,000
Melons	75,000
Parsnips	75,000
Potatoes	75,000
Eggplants	50,000
Okra	50,000
Summer squash	50,000
Sweet corn	50,000
forage	50,000
Sweet potatoes	50,000
Tomatoes	50,000
Pineapples	40,000
Cucumbers	30,000
Lettuce	30,000
Peppers	30,000
Cottonseed	25,000
Peanuts[c]	25,000
Asparagus	10,000
Cauliflower	10,000
Lima beans	5,000
Strawberries	5,000
Quarantine fumigation uses[d]	
Beans, string	10,000
Bitter melons	10,000
Cantaloupes	10,000
Cavendish bananas	10,000
Cucumbers	10,000
Guavas	10,000
Litchi fruit	10,000
Litchi nuts	10,000
Longan fruit	10,000
Mangoes	10,000
Peppers, bell	10,000
Pineapples	10,000
Zucchini squash	10,000
Cherries[e]	25,000
Plums (fresh prunes)[e]	25,000

TABLE IV-3 (cont.)

Commodity	Tolerance (μg/kg)
Food additive tolerances	
Milled grain fractions[f]	125,000
Fermented malt beverages from corn grits and cracked rice[g]	25,000

[a] Source: U.S. EPA, 1983; 49 FR 22082; 49 FR 17144

[b] Inorganic bromides (calculated as Br) (40 CFR 180.126)

[c] 40 CFR 180.126(a) prohibits the use of treated peanut hay and hulls as feed for meat and dairy animals.

[d] Inorganic bromides (calculated as Br) (40 CFR 180.146), unless otherwise specified

[e] Total inorganic and organic bromides (40 CFR 180.146)

[f] Inorganic bromides (calculated as Br) from all fumigation sources including grain mill machinery (21 CFR 193.225)

[g] Total inorganic bromides (calculated as Br) from all fumigations with bromine compounds [21 CFR 193.230(d)]

The monitoring data available are not sufficient to determine regional variations in levels of exposure to EDB. However, urban and industrial atmospheres appear to contain higher levels of EDB, as expected due to use in leaded gasoline.

The daily respiratory intake of EDB from air was estimated using the assumptions presented in Table IV-4 and the median and maximum levels for EDB reported above. The estimates in Table IV-4 indicate that the daily EDB intake from air ranges from 0.0-79 μg/kg/day. The values presented do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

Summary

The data obtained on the intake of EDB from drinking water, food and ambient air were insufficient for use in determining which of the three sources is the major contributor to total intake. However, ranges of total intake for the three sources were calculated from the ranges of intakes estimated for drinking water and air, and from the estimated dietary intake of 0.0066 μg/kg/day. Total intake from the three sources is estimated to range from 0.0066-95 μg/kg/day. No data on acceptable daily intakes for EDB were obtained with which to compare this intake range.

TABLE IV-4

Estimated Respiratory Intake of EDB*

Air Concentration (μg/m³)	Intake (μg/kg/day)
0.0	0.0
0.2	0.066
1.5	0.49
240	79

*Assumptions: 70 kg male inhaling 23 m³ of air/day (ICRP, 1975)

References

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V. HEALTH EFFECTS IN ANIMALS

General Toxicity

Acute Toxicity.

Oral Exposure -- Limited data are available regarding the acute toxicity of orally administered EDB in laboratory animals. The design and results of the available studies are detailed in Table V-1 and summarized below.

Single-dose oral $LD_{50}s$ of 146 and 117 mg EDB/kg bw have been determined for male and female rats of unspecified strain, respectively (Rowe et al., 1952). Single-dose oral $LD_{50}s$ of 420, 55 and 110 mg/kg bw were determined for female mice, female rabbits and mixed sex guinea pigs, respectively, in the same study. Toxic effects other than lethality were not described in these species, but these data indicate that rabbits are more sensitive and mice are less sensitive than rats to orally administered EDB. In a series of studies on the hepatic effects of orally administered EDB, Nachtomi and coworkers (Nachtomi et al., 1968; Nachtomi and Alumot, 1972; Broda et al., 1976; Nachtomi, 1980) administered single doses of 110-120 mg EDB/kg bw to male or female rats of various strains (Albino, Wistar or unreported strains). Mortality observations were not reported in these studies, but the results of the Rowe et al. (1952) study, in which a similar vehicle (olive oil) and method of administration (gavage) was used, suggest that these doses were near lethal levels. As detailed in Table V-1, biochemical indices of liver damage included the following: initially decreased and subsequently increased levels of free sulfhydryl groups, increased levels of triglycerides and total lipids, increased thymidine incorporation into DNA, increased liver and blood AP, increased SGPT and

TABLE V-1

Acute Oral Toxicity of EDB

Species/	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Rat/NR	M/60	~99% (commercial)	olive oil/ acacia	146 mg/kg, gavage, single dose	14-day LD_{50}[a]	Rowe et al., 1952
	F/40	~99% (commercial)	olive oil/ acacia	117 mg/kg, gavage, single dose	14-day LD_{50}[a]	Rowe et al., 1952
Rat/Albino	M/10, F/16	NR	soybean oil	120 mg/kg, gavage, single dose	Significant increase in relative liver weight, liver AP content, and relative adrenal weight after 20 hours. Adrenal ascorbic acid content and blood AP significantly increased in females but not males, but liver NADP and ascorbic acid content unchanged in either sex. Above parameters unchanged 4-5 hours after treatment. Drop in the level of free sulfhydryl groups in the liver in the first 2 hours in both sexes, followed by elevated level after 18-20 hours.	Nachtomi et al., 1968
Rat/Albino	M/2-5 per sacrifice	NR	olive oil	0 or 110 mg/kg, gavage, single dose	Rats sacrificed 2, 4, 8, 12 or 22 hours after treatment. Significantly ($p<0.05$) increased levels of triglycerides and total lipids[b] were found in the liver in 12/22 and 22 hours after treatment, respectively. Mortality observations not reported.	Nachtomi and Alumot, 1972
Rat/Albino	M/6-8 per sacrifice	NR	olive oil	0 or 110 mg/kg, gavage, single dose	Rats sacrificed 2, 4, 8, 12, 17 and 22 hours after treatment, sinusoidal dilations and centrilobular necrosis in the liver began after 8 hours. Other tissues not examined and mortality observations not reported.	Broda et al., 1976
Rat/Wistar	M/3-9 per dose and sacrifice	NR	corn oil	0, 75, 100, 150 or 220 mg/kg, gavage, single dose	Rats sacrificed at different doses and time intervals in multiple experiments. Liver DNA strand breaks indicated by slow sedimentation in alkaline sucrose gradients at all doses after 2-4 hours. Experiments with 220 and 75 mg/kg showed that the damage persisted at least 24 hours and was repaired by 96 hours, respectively. Mortality observations not reported.	Nachtomi and Sarma, 1977

TABLE V-1 (cont.)

Species/	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Rat/Wistar	M/3-11 per dose and sacrifice	NR	corn oil	0, 75 or 100 mg/kg, gavage, single dose	Rats sacrificed at different doses and time intervals in multiple experiments. Induction of hepatic DNA synthesis indicated by increased relative liver weight, increased DNA content, increased thymidine incorporation into DNA, higher mitotic index, and autoradiographic examination. Histological evidence of necrosis not found at either dose up to 5 days after treatment, and fatty changes or other alterations of hepatocytes "rarely" seen. Mortality observations not reported.	Nachtomi and Farber, 1978
Rat/NR	M/3-6 per sacrifice	NR	corn oil	0 or 110 mg/kg, gavage, single dose	Rats fasted overnight before treatment. Thymidine incorporation into liver DNA, SGPT and serum SDH significantly ($p<0.05$) elevated 48 hours after treatment in rats that were kept fasting; thymidine incorporation also significantly increased 24 hours after treatment in rats that were kept fasting. Thymidine incorporation and liver weight significantly increased after 24 and 48 hours and SDH significantly increased after 48 hours in rats that were refed 24 and 48 hours after treatment.	Nachtomi, 1980
Rat/NR	M/8-15 per group	NR	corn oil	0 or 110 mg/kg, gavage, single dose	Thymidine incorporation into liver DNA significantly ($p<0.05$) increased. Diethyldithiocarbamate (DDC) enhanced EDB toxicity as indicated by significantly elevated serum GPT, serum AP, liver, AP, liver Ca^{2+} and thymidine incorporation in DDC-pretreated rats.	Nachtomi, 1980
Rat/NR	M/6-8 per dose	NR	diet	0, 100 or 200 ppm in the diet (0, 100 or 200 mg/kg feed, reportedly equal to ~10-20 mg/kg bw) for 18 days	Thymidine injected daily. No significant increase in liver weight, liver DNA content or thymidine incorporation.	Nachtomi, 1980

TABLE V-1 (cont.)

Species/	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Rat/NR	M/10	NR	oil	0 or 10 mg/kg/day, gavage, for 12 days	Serum GPT and SDH significantly ($p<0.05$) elevated. Daily administration of phenobarbitone (PB) prior to EDB treatment protected against GPT and SDH serum enzyme elevation. Similar pretreatment with DDC enhanced EDB toxicity as indicated by significantly ($p<0.05$) increased liver weight, serum GPT and thymidine incorporation in DNA.	Nachtomi, 1980
Mouse/ Carworth	F/20	NR	olive oil	420 mg/kg, gavage, single dose	14-day LD_{50}	Rowe et al., 1952
Rabbit/NR	F/55	NR	olive oil/ acacia	55 mg/kg, gavage, single dose	14-day LD_{50}	Rowe et al., 1952
Guinea pig/NR	mixed/40	NR	olive oil/ acacia	110 mg/kg, gavage, single dose	14-day LD_{50}[c]	Rowe et al., 1952
Dog/NR	NR/1	NR	water	0.55 cc/kg (~550 mg/kg), gavage, single dose	CNS effects indicated by vomiting, defecation, "depression," increased salivation, sleepiness. Death within 20 hours.	Kistler and Luckhardt, 1929
Dog/NR	NR/1	NR	water	0.25 cc/kg (~250 mg/kg), gavage, single dose	CNS effects similar to 0.55 cc/kg dose. Death within 20 hours.	Kistler and Luckhardt, 1929
Dog/NR	NR/1	NR	water	0.0625 cc/kg (~62.5 mg/kg), gavage, single dose	Vomiting and "depression." Death within 22 hours.	Kistler and Luckhardt, 1929

[a] The LD_{50}s for the male and female rats in the Rowe et al. (1952) study differed significantly ($p<0.05$).

[b] Conjugated double bond (diene) formation in the microsomal liver lipid, indicating lipid peroxidation

[c] LD_{50} data for the male and female guinea pigs were not reported separately because the difference was not statistically significant.

SDA. Pathological indices included: increased relative liver weight, sinusoidal dilation and centrilobular necrosis. This evidence of liver damage was apparent 8-24 hours after EDB administration. Related studies with rats showed that consistent hepatic effects (liver DNA damage and repair as indicated by slow sedimentation in alkaline sucrose gradients, increased DNA content, increased thymidine incorporation into DNA, elevated mitotoic index and increased relative liver weight) were produced by single oral doses of 75 mg EDB/kg bw (Nachtomi and Sarma, 1977; Nachtomi and Farber, 1978). Histological evidence of necrosis was not noted up to 5 days after administration of 75 or 100 mg/kg bw, and fatty changes and other unspecified alterations of hepatocytes were "rarely" seen (Nachtomi and Farber, 1978).

Significant increases in liver weight, liver DNA content or thymidine incorporation into liver DNA were not produced in rats by dietary administration of 100 or 200 ppm EDB for 18 days (reportedly equivalent to ~10-20 mg EDB/kg bw/day), but administration of 10 mg EDB/kg bw/day by gavage for 12 days elicited significantly ($p<0.05$) increased levels of SGPT and SDH (Nachtomi, 1980).

Symptoms of toxicity and effects on tissues other than the liver were not described in the above acute oral studies with rats. However, a limited oral study with dogs that investigated the pharmacological properties of EDB indicated that CNS depression is a primary effect of lethal exposures (Kistler and Luckhardt, 1929). It was observed that salivation, vomiting, sleepiness, and other unspecified symptoms of depression and then death was elicited by doses in the range of ~62-550 mg/kg bw, but these effects cannot be associated with the reported doses because vomiting occurred and only one dog per dose was tested (see Table V-1).

Inhalation Exposure -- Rowe et al. (1952) exposed groups of 5-30 rats of both sexes (unspecified strain from an in-house stock colony) to concentrations of 100, 200, 400, 800, 1600, 3000, 5000 or 10,000 ppm EDB for durations ranging from 0.02-16.0 hours (Table V-2). The purpose of the experiment was to graph the data to estimate single exposures expected to cause 0.01, 50 and 99.99% mortality of rats. As detailed in Table V-2, the maximum nonfatal exposures were 1.2 minutes at 10,000 ppm, 2.4 minutes at 5000 ppm, 6 minutes at 3000 ppm, 12 minutes at 1600 ppm, 36 minutes at 400 ppm, 2 hours at 200 ppm and 16 hours at 100 ppm. EDB reportedly produced only slight anesthetic effects at the concentrations used, but central nervous system depression (not elaborated) was observed in rats exposed at the higher concentrations (not specified). Deaths generally occurred within 24 hours at the higher concentrations and were caused by respiratory or cardiac failure. Deaths resulting from exposures at lower concentrations (unspecified) typically were delayed, occasionally for as long as 12 days after exposure, and were usually due to pneumonia that developed as a result of lung injury. The rats that survived exposure at the lower concentrations exhibited a similar progression of toxic signs (e.g., weight loss, rough and unkempt appearance, irritability, bloody nasal discharge). Histopathologic examinations were conducted on the lungs, heart, liver, kidneys, spleen, testes, pancreas and adrenals from rats that were sacrificed 16-24 hours after exposure to lethal concentrations of EDB; effects were found in the lungs (congestion, edema, hemorrhages, inflammation, increased weight),

TABLE V-2

Acute Inhalation Toxicity of EDB

Species/ Strain	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Rat/NR	mixed/20	~100%[a]	vapor	10,000 ppm x 0.1 hour (77 mg/ℓ)	20/20 died	Rowe et al., 1952
	mixed/10	~100%[a]	vapor	10,000 ppm x 0.07 hour	7/10 died	
	mixed/20	~100%[a]	vapor	10,000 ppm x 0.02 hour	0/20 died	
	mixed/20	~100%[a]	vapor	5000 ppm x 0.14 hour (38.5 mg/ℓ)	20/20 died	
	mixed/15	~100%[a]	vapor	5000 ppm x 0.07 hour	5/15 died	
	mixed/20	~100%[a]	vapor	5000 ppm x 0.04 hour	0/20 died	
	mixed/10	~100%[a]	vapor	3000 ppm x 0.20 hour (23.1 mg/ℓ)	5/10 died	
	mixed/20	~100%[a]	vapor	3000 ppm x 0.10 hour	0/20 died	
	mixed/20	~100%[a]	vapor	1600 ppm x 0.50 hour (12.3 mg/ℓ)	20/20 died	
	mixed/15	~100%[a]	vapor	1600 ppm x 0.30 hour	4/15 died	
	mixed/30	~100%[a]	vapor	1600 ppm x 0.20 hour	0/30 died	
	mixed/20	~100%[a]	vapor	800 ppm x 0.80 hour (6.16 mg/ℓ)	13/20 died	
	mixed/20	~100%[a]	vapor	800 ppm x 0.40 hour	4/20 died	
	mixed/20	~100%[a]	vapor	400 ppm x 5.0 hours (3.08 mg/ℓ)	20/20 died	
	mixed/25	~100%[a]	vapor	400 ppm x 2.0 hours	16/25 died	
	mixed/20	~100%[a]	vapor	400 ppm x 0.8 hour	1/20 died	
	mixed/20	~100%[a]	vapor	400 ppm x 0.6 hour	0/20 died	
	mixed/20	~100%[a]	vapor	200 ppm x 16 hours (1.54 mg/ℓ)	19/20 died	

TABLE V-2 (cont.)

Species/ Strain	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Rat/NR	mixed/20	~100%^{a}	vapor	200 ppm x 8.5 hours	9/20 died	Rowe et al., 1952
	mixed/11	~100%^{a}	vapor	200 ppm x 3.0 hours	1/11 died	
	mixed/5	~100%^{a}	vapor	200 ppm x 2.0 hours	0/5 died	
	mixed/20	~100%^{a}	vapor	200 ppm x 1.4 hours	0/20 died	
	mixed/20	~100%^{a}	vapor	100 ppm x 16.0 hours (0.77 mg/ℓ)	0/20 died	
	mixed/20	~100%^{a}	vapor	100 ppm x 12.0 hours	0/20 died	
	mixed/20	~100%^{a}	vapor	100 ppm x 8.5 hours	0/20 died	
	F/10	~100%^{a}	vapor	800 ppm x 0.15 hour (6.16 mg/ℓ)	Adverse hepatic effects^b	
	F/10	~100%^{a}	vapor	800 ppm x 0.10 hour	No adverse hepatic effects^b	
	F/10	~100%^{a}	vapor	200 ppm x 1.0 hour (1.54 mg/ℓ)	Adverse hepatic effects^b	
	F/10	~100%^{a}	vapor	200 ppm x 0.7 hour	No adverse hepatic effects^b	
	F/10	~100%^{a}	vapor	100 ppm x 4.0 hours (0.77 mg/ℓ)	Adverse hepatic effects^b	
	F/10	~100%^{a}	vapor	100 ppm x 2.5 hours	No adverse hepatic effects^b	
	F/10	~100%^{a}	vapor	50 ppm x 7.0 hours (0.38 mg/ℓ)	No adverse hepatic effects^{b,c}	
	F/10	~100%^{a}	vapor	100 ppm x 7 hours/day 7 exposures in 9 days	Four unexposed controls used. 3/10 died after 1, 5 and 7 exposures. Survivors showed blood-tinged stomach contents, significantly increased lung, liver and kidney weights, thickening of alveolar walls with slight leukocytic infiltration, cloudy swelling of liver (no fatty degeneration) and slight congestion and hemosiderosis of the spleen. No effect on heart or spleen weights or blood nonprotein nitrogen, urea nitrogen or plasma prothrombin clotting time.	

TABLE V-2 (cont.)

Species/ Strain	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Rats/NR	F/18	~100%^{a}	vapor	50 ppm x 7 hours/day 12 exposures in 16 days	Increased liver and kidney weights, but no significant histological effects in these organs. No effect on BUN or total liver lipid content.	Rowe et al., 1952
	F/8	~100%^{a}	vapor	25 ppm x 7 hours/day 13 exposures in 16 days	No adverse effects as judged by mortality, appearance or behavior, growth, body or organ weights, BUN, hematology, total liver lipid or histopathology.	
Guinea pig/NR	NR/3	commercial grade	vapor	0.8% x 0.5 hour (~8000 ppm)	Death after 6-18 hours. Pulmonary congestion and edema. Parenchymal degeneration and interstitial edema in abdominal organs as detailed in text. Degeneration of the renal tubule loops was the most pronounced lesion.	Thomas and Yant, 1927
	NR/3	commercial grade	vapor	0.4% x 1.0 hour (~4000 ppm)	Death after 6-18 hours with effects as described above.	
	NR/3	commerical	vapor	0.2% x 1.0 hour (~2000 ppm)	Death after 6-18 hours with effects as described above.	
Guinea pig/NR	mixed/20	~100%^{a}	vapor	400 ppm x 7.0 hours (3.08 mg/ℓ)	20/20 died	Rowe et al., 1952
	mixed/20	~100%^{a}	vapor	400 ppm x 5.0 hours	18/20 died	
	mixed/10	~100%^{a}	vapor	400 ppm x 3.0 hours	5/10 died	
	mixed/20	~100%^{a}	vapor	400 ppm x 2.0 hours	0/20 died	
	mixed/15	~100%^{a}	vapor	200 ppm x 7.0 hours (1.54 mg/ℓ)	0/15 died	
	F/7	100%	vapor	50 ppm x 7 hours/day 13 exposures in 17 days	Depressed weight gain but no effect on organ weights, BUN, total liver lipid content, or gross or histopathology.	
	F/8	100%	vapor	25 ppm x 7 hours/day 13 exposures in 17 days	No evidence of adverse effects as judged by mortality, behavior, growth, body or organ weights, BUN or histopathology.	

TABLE V-2 (cont.)

Species/ Strain	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Rabbit/NR	F/4	~100%	vapor	100 ppm x 7 hours/day x 4 days	3/4 died after 2-3 exposures with widespread central fatty degeneration and some necrosis in the liver.	Rowe et al., 1952
	F/2	~100%	vapor	100 ppm x 7 hours/day x 2 days	Body weight loss but no effect on organ weights. BUN and nonprotein nitrogen normal. Slight cloudy swelling of the liver.	

[a] Commercial product purified by redistillation

[b] Increased liver weight and "slight" histopathological changes in the liver were used as evaluation criteria

[c] Effect exposure at 50 ppm not determined

liver (cloudy swelling, centrilobular fatty degeneration and necrosis) and kidneys (slight interstitial congestion and edema, occasional slight cloudy swelling of the tubular epithelium).

Additional exposures were conducted with groups of 10 female rats at concentrations of 50, 100, 200 or 800 ppm to determine the most severe single exposures that did not produce evidence of toxic injury (Rowe et al., 1952). The range of exposure durations tested were not specified, but the hours of exposure with and without adverse effect, respectively, as indicated by increased liver weight and "slight" histopathological changes in the liver, were estimated to be 2.5 and 4.0 hours at 100 ppm, 0.7 and 1.0 hours at 200 ppm, and 0.1 and 0.15 hours at 800 ppm. Exposure to 50 ppm EDB for 7.0 hours did not elicit liver effects, but an exposure that elicited effects at 50 ppm was not determined.

Rowe et al. (1952) also exposed groups of 8-18 female rats 7 hours/day to EDB at 25 ppm (13 exposures in 16 days), 50 ppm (12 exposures in 16 days) or 100 ppm (7 exposures in 9 days). Appearance, behavior, body and organ weights and blood urea nitrogen content were determined. Histological examinations were performed on the lungs, heart, liver, kidneys, spleen, testes, pancreas and adrenals. Groups of matched unexposed and air-exposed rats were used as controls, and additional parameters that were examined are indicated in Table V-2. Mortality (3/10), increased organ weights (lung, liver and kidneys) and slight histological alterations (thickening of alveolar wall, cloudy swelling in liver, congestion in spleen) were found at 100 ppm, but the only effects noted at 50 ppm were increased liver and kidney weights. Adverse effects were not noted at 25 ppm. This progression of effects is consistent with the responses observed in rats exposed to the same concentrations in subchronic experiments in the same study (see Inhalation Studies Section and Table V-5).

A limited number of single exposures that were conducted with 200 and 400 ppm EDB suggest that guinea pigs are less susceptible to lethal effects than rats exposed at the same concentration (Rowe et al., 1952) (see Table V-1). At 200 ppm, exposure for 7 hours produced death in 0/15 guinea pigs and 4/11 rats. At 400 ppm, exposures for 2, 3 or 7 hours produced death, respectively, in 0/20, 5/10 and 18/20 of the guinea pigs and 16/25, 17/20, and 20/20 of the rats. Symptoms of toxicity and pathological effects of EDB exposure were not specifically described for the guinea pigs.

Groups of seven guinea pigs that were exposed repeatedly to 50 ppm EDB (thirteen 7-hour exposures in 17 days) showed only depressed weight gain, and adverse effects were not apparent in eight guinea pigs that were similarly exposed to 25 ppm EDB (Rowe et al., 1952) (see Table V-2). The toxicity endpoints that were evaluated in this study are essentially the same as those described previously for rats.

In rabbits, 3 of 4 died after several daily 7-hour exposures to 100 ppm EDB with degenerative and necrotic alterations in the liver (Rowe et al., 1952) (see Table V-2).

Several studies were conducted on the toxicity of inhaled EDB to different species of animals during the late 1920s (Thomas and Yant, 1927; Lucas, 1928; Kochmann, 1928; Glaser and Frisch, 1929; Kistler and Luckhardt, 1929;

Merzbach, 1929). These studies used rabbits, cats, guinea pigs and/or dogs subjected to single or a limited number of repeated exposures that in many cases resulted in death, but are not detailed here because of inadequate experimental design and/or results. The effects of exposure that were described in these reports are consistent with those detailed previously, and include respiratory irritation (e.g., rhinitis, pneumonic changes), CNS dysfunction (e.g., excessive salivation, locomotor impairment, spasms, paralysis of the extremities, increased respiration) and pathologic changes in the lungs (e.g., congestion and edema), GI tract, liver, kidney and other abdominal organs (e.g., enlargement and degenerative changes).

Dermal Exposure -- Dermal studies with small numbers of rats (Thomas and Yant, 1927) and rabbits (Rowe et al., 1952) have shown that EDB can be absorbed in lethal amounts through intact skin. Application of single doses in the ranges of 0.25-1.0 cc/rat (~1.5-6 g/kg, assuming a body weight of 0.35 kg) and 0.2-1.1 g/kg rabbit elicited local and systemic toxic effects (e.g., erythema, edema, CNS depression) as well as death as detailed in Table V-3. The experiments of Rowe et al. (1952) indicated that the local response was more severe when EDB was confined to the skin (i.e., occluded application).

Subchronic and Chronic Toxicity.

Oral Exposure -- NCI (1978) conducted a 6-week gavage study with Osborne-Mendel rats and $B6C3F_1$ mice for the purpose of establishing maximum tolerated dosages to be used in a chronic carcinogenicity bioassay (see Carcinogenicity Section).

TABLE V-3

Acute Dermal Toxicity of EDB

Species/ Strain	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Rat/NR	NR/2	commercial (1 rat) purified (1 rat)	NR	1 cc/2 cm^2 of shaved abdomen (~6 g/kg)	Death after 6-18 hours. Marked cutaneous hyperemia, contracted abdominal muscles, pronounced weakness.	Thomas and Yant, 1927
	NR/2	commercial (1 rat) purified (1 rat)	NR	0.5 cc/2 cm^2 of shaved abdomen (~3 g/kg)	Death after 6-18 hours with effects as described above.	
	NR/2	commercial (1 rat) purified (1 rat)	NR	0.25 cc/2 cm^2 of shaved abdomen (~1.5 g/kg)	Death after 6-18 hours with effects as described above.	
Rabbit/NR	NR/NR	~99% (commercial)	undiluted	unspecified volume applied to inner surface of ear and shaven belly	Marked erythema and edema when occluded for a "few hours." Slight erythema when not occluded.	Rowe et al., 1952
	NR/5	~99% (commercial)	undiluted	1.1 g/kg x 24 hours, occluded trunk (Draize method)	5/5 died within 4 days. Local erythema, edema, and necrosis. CNS depression and reduced body temperature.	
	NR/5	~99% (commercial)	undiluted	0.65 g/kg x 24 hours, occluded trunk (Draize method)	4/5 died within 4 days. Effects as described above.	
	NR/5	~99% (commercial)	undiluted	0.30 g/kg x 24 hours, occluded trunk (Draize method)	2/5 died within 4 days. Effects as described above.	
	NR/15	~99% (commercial)	undiluted	0.21 g/kg x 24 hours, occluded trunk (Draize method)	1/15 died within 4 days. Effects as described above.	
	NR/NR	~99% (commercial)	butyl carbitol acetate	1.0% solution applied to ear and occluded shaven belly, 10 times in 14 days (volume not stated)	Slight irritation of ear (erythema and exfoliation). Marked irritation of belly (erythema and edema progressing to necrosis and exfoliation. Healing without scarring within 7 days after treatment.	

NR = Not reported

EDB (>99% purity) was administered in corn oil to groups of 10 animals (5/sex) at dosages of 0 (vehicle control), 40, 63, 100, 159 or 251 mg/kg bw/day (mice), and 0, 40, 63, 100, 163 or 251 mg/kg bw/day (rats), 5 consecutive days/week for 6 weeks. The animals were observed for an additional 2 weeks to detect any delayed toxicity. The results of this study were incompletely reported; the only endpoints evaluated were mortality and body weight depression.

None of the rats in the 63 mg/kg group died during the 8-week period, but 1 male and 1 female died at 100 mg/kg (NCI, 1978) (Table V-4). The mean body weight for the male and female rats at 100 mg/kg were 75 and 82% of the control value, respectively, but the weights of the rats at ≤63 mg/kg were reportedly within 10% of the control group weight. Mortality and body weight data for rats exposed to dosages >100 mg/kg were not reported.

All of the male mice that were administered dosages of ≤159 mg/kg survived. Mean body weight in the 63 and 159 mg/kg males was 71 and 93% of the control value, respectively (NCI, 1978); mortality and body weight data for the 251 mg/kg males were not reported. All female mice survived except 1 in the 100 mg/kg and 2 in the 251 mg/kg groups. Body weight was apparently not significantly affected by exposure (details not provided).

Aman et al. (1946) inadequately reported the experimental design and results of a subchronic oral toxicity study that was conducted with rats and guinea pigs. Nineteen animals were fed EDB in oil or 50% aqueous alcohol over a period of ~4 months. Maximum average daily doses of ~40-50 mg/kg reportedly did not elicit any abnormal signs (signs other than body weight

TABLE V-4

Subchronic/Chronic Oral Toxicity of EDB

Species/ Strain	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Rat/ Osborne-Mendel	mixed/10[a]	>99% (technical)	corn oil	0 (vehicle control), 40, 63, 100, 163 or 251 mg/kg/day, by gavage, 5 days/week for 6 weeks. 2-week observation period.	Range-finding study for a chronic bioassay; results incompletely reported. No mortality at 63 mg/kg/day but 1 rat/sex died at 100 mg/kg/day. Mean body weight for the males and females at 100 mg/kg/day was 75 and 82% of control values, respectively; mean weight at lower doses was ≥10% of control values.	NCI, 1978
	M/20 or 50[b]	>99% (technical)	corn oil	0 (vehicle control), 27.4 and 29.1 mg/kg/day by gavage for 49 weeks.[c] Untreated observation period with control (2 weeks) but not with treated rats.	Carcinogenicity bioassay. Dose-related weight depression and early mortality as detailed in text, but mortality may have been tumor-related (forestomach carcinoma). Nonneoplastic findings in the control, low- and high-dose females included forestomach hyperkeratosis (0/20, 4/50, 18/50), forestomach acanthosis (0/20, 4/50, 18/50), peliosis hepatis (0/20, 1/47, 5/48), liver inflammation (0/20, 4/47, 2/48), adrenal cortical degeneration (1/20, 3/44, 8/45).	
	F/20 or 50[b]	>99% (technical)	corn oil	0 (vehicle control), 26.7 or 28.1 mg/kg/day by gavage for 49 weeks.[c] Untreated observation period with control (2 weeks) but not with treated rats.	As detailed above	
Rat/NR Guinea pig/NR	NR/NR	NR	unspecified oil or 50% aqueous alcohol	40-50 mg/kg, average daily dose by gavage for ~4 months	Design and results inadequately and ambiguously reported. 19 animals used in study, but numbers of each species, range of dosages and exposure schedule not reported. No effect on body weight or other unspecified signs of toxicity. Pathologic examinations not conducted.	Aman et al., 1946

TABLE V-4 (cont.)

Species/ Strain	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Mouse/ B6C3F$_1$	mixed/10[a]	NR	unspecified oil or 50% aqueous alcohol	0 (vehicle control), 40, 63, 100, 159 and 251 mg/kg/day, 5 days/ week for 6 weeks. 2-week observation period	Range-finding study for a chronic bioassay; results incompletely reported. No mortality in males below 159 mg/kg/ day; all females survived except 1 at 100 mg/kg/day and 2 at 251 mg/kg/day. Body weight reduced 71 and 91% in males at 63-159 mg/kg/day; no effect in females.	NCI, 1978
	M/20 or 50[b]	NR	unspecified oil or 50% aqueous alcohol	0 (vehicle control), 43.6 and 76.6 mg/kg/day by gavage for 53 weeks.[c] Untreated observation periods of 6 (control), 25 (low-dose) and 24 (high-dose) weeks.	Carcinogenicity bioassay. Dose-related weight depression and early mortality as detailed in text, but mortality may have been tumor-related (forestomach carcinoma). Non-neoplastic findings in the control, low- and high-dose males included forestomach hyperkeratosis (0/20, 0/50 and 13/50), forestomach acanthosis (0/50, 1/50, 5/50) and testicular atrophy (0/20, 0.45, 10/47).	
	F/20 or 50[b]	NR	unspecified oil or 50% aqueous alcohol	0 (vehicle control), 43.9 and 76.6 mg/kg/day by gavage for 53 weeks. Untreated observation periods of 7 (control), 37 (low-dose) and 25 (high-dose) weeks.	Dose-related weight depression and early mortality as detailed in text, but mortality may have been tumor-related (forestomach carcinoma). Non-neoplastic findings in the control, low- and high-dose females include forestomach hyperkeratosis (0/20, 1/49 and 12/50) and forestomach acanthosis (0/20, 0/49 and 9/50).	

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[a] 5 per sex per dose tested

[b] Groups of 20 control and 50 treated were used

[c] TWA doses. Exposure schedule detailed in Carcinogenicity Section.

not indicated). The number of rats and guinea pigs tested and the range of doses and exposure schedule were not specified. It was indicated in a table that summarized body weight data for selected animals (1 control and 3 treated rats, 1 treated and 1 control guinea pig) that an average daily dose as low as 17 mg/kg (period of feeding 95 days) had no effect on body weight in one rat, and that another rat that was fed 20 mg of EDB daily (body weight not reported) died after 3 months. It was not stated whether pathologic examinations were conducted in this study.

A chronic carcinogenicity bioassay of EDB has been conducted with Osborne-Mendel rats and B6C3F$_1$ mice that were treated by gavage (NCI, 1978). Groups of 50 rats were intubated 5 consecutive days/week at TWA dosages of 27.4 and 29.1 mg/kg bw/day for 49 weeks (males) or 26.7 and 28.1 mg/kg bw/day for 61 weeks (females). The exposure schedule is detailed in the Other Effects Section, but it should be noted here that the initial dosages differed significantly in low- and high-dose groups (28.6 and 57.1 mg/kg/day, respectively); early deaths of high-dose male and female rats prompted discontinuation of dosing during weeks 17-29. There were no untreated observation periods for the dosed rats after treatment. Groups of 50 mice of each sex were intubated similarly at TWA dosages of 43.9 and 76.6 mg/kg bw/day for 53 weeks, and observed for 25 (low-dose males), 24 (high-dose males), 37 (low-dose females) and 25 weeks (high-dose females). Groups of 20 rats and mice of each sex per dose were employed as vehicle (corn oil) controls. Comprehensive histopathologic examinations were conducted on all treated and control animals. Neoplastic findings are summarized in the Other Effects Section.

Treatment-related mean body weight depression reportedly was apparent in the low- and high-dose rats of both sexes after the first 10 weeks of the study (NCI, 1978); examination of growth curves shows that the weights of the low-dose males, high-dose males, low-dose females and high-dose females were ~15, 15, 10 and 15% lower than control values, respectively, at the end of the treatment periods. The Tarone test for positive association between increased dosage and accelerated mortality was significant ($p<0.001$) for both male and female rats. The study was originally scheduled to last 110 weeks but high mortality led to early termination; mortality was 31/50 and 45/50 in the low- and high-dose males, respectively, in week 49 and 48/50 and 49/50 in the low- and high-dose females, respectively, in week 61. Clinical observations included reddened ears and a hunched appearance in all treated rats by week 5. Firm distended abdomens and abdominal urine stains were noted by week 38.

A high incidence of early-developing squamous cell carcinomas in the forestomach was observed in the treated but not in control rats, suggesting that the mortality may have been tumor-related (see Table V-4). Most rats that survived beyond week 15 showed evidence of this carcinoma. Historical pooled controls in the past have shown a low incidence (0.1%) of stomach tumors in male and female Osborne-Mendel rats, which indicates the present experiment was well controlled. Lesions included hyperkeratosis and acanthosis of the forestomach in the high-dose rats of both sexes (see Table V-4). These lesions were considered to be part of a pattern of accelerated growth in the forestomach. Degenerative changes in the liver (peliosis hepatitis and inflammation, which was not otherwise specified) and adrenal gland (cortical cell degeneration) also were found in small numbers of treated rats of both sexes (see Table V-4).

Dose-related mean body weight depression reportedly was apparent in both male and female mice from week 10 through the remainder of the study (NCI, 1978); examination of growth curves shows that the weights of the low-dose males, high-dose males, low-dose females and high-dose females were ~5, 20, 5 and 25% lower than control values, respectively, during the terminal treatment periods. The Tarone test for positive assocation between dosage and mortality was significant ($p<0.001$) for both male and female mice; mortality was 2/20, 30/50 and 40/50, respectively, in the vehicle control, low-dose and high-dose males after 58 weeks, and 1/20, 22/50 and 42/50, respectively, in the vehicle control, low-dose and high-dose females after 70 weeks. Clinical observations included alopecia, soft feces and body sores in all treated mice by week 14, and then hunched appearance (high-dose groups) and small inguinal nodules (low-dose males) as the study progressed. A high incidence of squamous cell carcinoma of the forestomach was observed in the treated mice but not in control mice (see Carcinogenicity Section), suggesting that the mortality may have been tumor-related. As with the rats, related non-neoplastic lesions in the forestomach (acanthosis and hyperkeratosis) were found in both male and female mice (see Table V-4). Testicular atrophy also occurred in the high-dose male mice.

Inhalation Studies -- A subchronic inhalation study was conducted with F344 rats and B6C3F$_1$ mice to determine the concentrations of EDB to be used in a chronic carcinogenesis bioassay (Reznik et al., 1980; NTP, 1982). In this study, groups of 4-5 male rats, 5-6 female rats, 10 male mice and 10 female mice were exposed to EDB (>99% pure) at vapor concentrations of 0, 3, 15 or 75 ppm for 6 hours/day, 5 days/week for 13 weeks. Animal weights were recorded weekly and comprehensive histopathological examinations were conducted at the end of the exposure period (Table V-5).

TABLE V-5

Subchronic/Chronic Inhalation Toxicity of EDB

Species/ Strain	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Rat/F344	M/4-5 per dose F/5-6 per dose	>99%	vapor	0, 3, 15 or 75 ppm, 6 hours/day, 5 days/week for 13 weeks	Range finding study for a chronic carcinogenicity bioassay. Dose-related depressed weight gain in males, but weight gain depressed in females only at 75 ppm. No mortality. Histological effects only at 75 ppm included swelling/vacuolation of the adrenal cortical cells (8/10), slightly decreased thyroid follicular size (6/10), and nasal cavity cytomegaly (8/10), focal hyperplasia (10/10), squamous metaplasia (8/10) and loss of cilia (10/10).	NTP, 1982; Reznik et al., 1980
Rat/F344	M/NR F/NR	NR	vapor	0, 23, 77 or 3076 mg/m^3 (0, 3, 10 or 400 ppm, respectively), 6 hours/day, 5 days/week for 13 weeks	Study summarized in PD 2/3 (U.S. EPA, 1980a). Decreased body weight gain, increased liver and kidney weights, and epithelial hyperplasia/squamous metaplasia of the nasal cavity at 400 ppm. Slight, reversible epithelial hyperplasia of the nasal cavity at 10 ppm. No consistent effect on any parameter (not elaborated) at 3 ppm.	Nitschke, 1980; U.S. EPA, 1980
Rat/NR	M/20 F/20	~100%	vapor	50 ppm x 7 hours/day 63 exposures in 91 days	10/20 males and 4/20 females died; death in males due to pneumonia and respiratory infections. No signficant effect on body weight but liver and kidneys weights increased; lung weight increased and testis weights decreased in males, and spleen weight increased in females. BUN, liver total lipid and hematological values normal. Evidence of old pneumonia in males, but no significant histological effects in the heart, liver, kidneys, spleen or testes.	Rowe et al., 1952
	F/18	~100%	vapor	50 ppm x 7 hours/day 12 exposure in 16 days	Increase liver and kidney weights but no effect on histology of these organs, BUN or total liver lipid content.	

TABLE V-5 (cont.)

Species/ Strain	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Rat/NR	M/20 F/20	~100%	vapor	25 ppm x 7 hours/day 151 exposures in 213 days	High mortality in males (10/20) due to pneumonia and respiratory infection, but not in females (3/20). No adverse effects indicated by appearance or behavior, growth, body or organ weights, BUN, hematology or histopathology.	Rowe et al., 1952
	F/8, F/15	~100%	vapor	25 ppm x 7 hours/day 13 exposures in 17 days	Two groups tested. No adverse effects indicated by above criteria or total liver lipid content.	
Rat/ Sprague-Dawley	M/48 per dose	99%	vapor	0 or 20 ppm, 7 hours/day, 5 days/week for 18 months	Carcinogenicity study. Mean body weight gain decreased and mortality increased at months 15 and 18; mortality at 0 and 20 ppm was ~2 and 10% and 63 and 90%, respectively. Hematologic parameters normal after 10-12 months of exposure, significantly elevated incidence of spleen atrophy (0/48 vs. 6/48), but testicular atrophy not found. Nasal cavity not examined. Neoplastic lesions induced as detailed in Carcinogenicity Section. Concurrent administration of 0.05% disulfiram in diet produced earlier and a higher percentage of deaths, depressed body weight gain from onset of treatment, low hematocrits, hemoglobin and RBC counts, and testicular atrophy in 90% of the rats.	Wong et al., 1982
Rat/ Sprague-Dawley	F/48 per dose	99%	vapor	0 or 20 ppm, 7 hours/day, 5 days/week for 18 months	Carcinogenicity study. Mean body weight depressed at month 18 and mortality increased at months 15 (6% controls, 40% treated) and 18 (13% controls, 77% treated). Hematologic parameters normal after 10-12 months of exposure. Non-neoplastic lesions not described but neoplastic lesions were induced as detailed in the Carcinogenicity Section. Nasal cavity not examined. Concurrent administration of 0.05% disulfiram in diet enhanced EDB toxicity as described for male rats.	Wong et al., 1982

TABLE V-5 (cont.)

Species/ Strain	Sex/No	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Rat/F344	M/50 per dose	99.3-99.4%	vapor	0 ppm for 104-106 weeks, 10 ppm for 103 weeks (1 week observation), 40 ppm for 88 weeks (0-1 week observation). 6 hours/ day, 5 days/week exposures	Carcinogenicity bioassay. Weight depression and increased mortality at high dose; terminal mortality was 12/50, 15/50 and 45/50. Limb and body weakness after week 52 at high dose. Non-neoplastic lesions in respiratory system (epithelial hyperplasia, squamous metaplasia or suppurative inflammation in the nasal cavity, trachea, bronchus or lung), liver (necrosis in 2/50, 6/50 and 19/50), kidney (toxic nephropathy) and testis (degeneration in 1/50, 10/50 and 18/49, atrophy, spermatic granulomas).	NTP, 1982
	F/50 per dose	99.3-99.4%	vapor	0 ppm for 104-106 weeks, 10 ppm for 103 weeks (1 week observation),40 ppm for 91 weeks (0-1 week observation). 6 hours/ day, 5 days/week exposures	Carcinogenicity bioassay. Weight depression and increased mortality at high-dose; terminal mortality was 12/50, 11/50 and 42/50. Limb and body weakness after week 52 at high dose. Non-neoplastic lesions in respiratory system (epithelial hyperplasia, squamous metaplasia and suppurative inflammation in the nasal cavity, trachea, bronchus or lung), liver (necrosis in 2/50, 3/50 and 13/50), kidney (toxic nephropathy at high dose), adrenal cortex (degeneration), and retina (atrophy).	
Mouse/ B6C3F$_1$	M/10 per dose F/10 per dose	>99%	vapor	0, 3, 15 or 75 ppm, 6 hours/day, 5 days/week for 13 weeks	Range-finding study for a carcinogenicity bioassay. Dose-related depressed weight gain in both sexes with mortality in 3 ppm males (4/10) and 75 ppm females (1/10). Eye irritation and nasal cavity cytomegaly (3/10 M, 9/10 F), focal hyperplasia (2/10 M, 8/10 F), squamous metaplasia (3/10 M, 9/10 F) and loss of cilia (3/10 M, 9/10 F) only at 75 ppm.	NTP, 1982
	M/50 per dose	99.3-99.4%	vapor	0 ppm for 79 weeks, 10 ppm or 40 ppm for 78 weeks (0-1 week observation) 6 hours/day, 5 days/week exposures	Carcinogenicity bioassay. Weight depression at high dose and increased mortality at low dose; terminal mortality was 27/50, 39/50 and 32/50. Poor survival not treatment-related (urinary infection). Limb and body weakness after week 52. Non-neoplastic lesions in respiratory system (epithelial hyperplasia in bronchus, serous/suppurative inflammation in nasal cavity).	

TABLE V-5 (cont.)

Species/ Strain	Sex/No.	Purity	Vehicle	Dose or Exposure	Effect/Comments	Reference
Mouse/ $B6C3F_1$	F/50 per dose	99.3-99.4%	vapor	0 ppm for 104-106 weeks 10 ppm for 103 weeks (1 week observation), 40 ppm for 90 weeks (0-1 week observation) 6 hours/day, 5 days/week exposures	Carcinogenicity bioassay. Weight depression at high dose and dose-related increased mortality; terminal mortality was 10/50, 31/50 and 43/50. Limb and body weakness after week 52. Non-neoplastic lesions in respiratory system (epithelial hyperplasia in nasal cavity, bronchus and lung, serous/suppurative inflammation in nasal cavity).	NTP, 1982
Guinea pig/NR	M/8 F/8	~100%	vapor	50 ppm x 7 hours/day 57 exposures in 80 days	Depressed growth but mortality not increased. Lung, liver and kidney weights increased but BUN normal. Slight central fatty degeneration in all livers, and slight interstitial congestion and edema with slight parenchymatous degeneration of tubular epithelium in 8/14 kidneys. No histological effects in lungs, heart, spleen, adrenal, pancreas or testes.	Rowe et al., 1952
Guinea pig/NR	F/7	~100%	vapor	50 ppm x 7 hours/day 13 exposures in 17 days	Depressed growth rate but no effect on above parameters.	Rowe et al., 1952
	M/8, F/8	~100%	vapor	25 ppm x 7 hours/day 145 exposures in 205 days	4/8 males and 2/8 females died from pulmonary infection, but no effect on behavior, growth, body or organ weights, BUN or histopathology.	
	F/8	~100%	vapor	25 ppm x 7 hours/day 13 exposures in 17 days	No evidence of adverse effects as judged by above criteria.	
Rabbit/NR	M/3, F/1	~100%	vapor	50 ppm x 7 hours/day 59 exposures in 214 days	No adverse effect indicated by appearance, behavior, growth, body or organ weights, BUN or histopathology.	Rowe et al., 1952
Monkey/NR	M/1, F/1	~100%	vapor	50 ppm x 7 hours/day 49 exposures in 70 days	Ill/nervous/unkempt appearance, depressed body weight gain, increased liver weight with central fatty degeneration and slightly increased total lipid content. Slight increase of kidney weight. Normal hematology and BUN, and no histopathologic effects in the lung, heart, kidney, spleen, adrenal, pancreas, lymph node, bone marrow, brain, muscle, urinary bladder or stomach	Rowe et al., 1952
Monkey/NR	M/1, F/1	~100%	vapor	25 ppm x 7 hours/day 156 exposures in 220 days	No adverse effects indicated by appearance, behavior, hematology, body or organ weights, BUN, total liver lipid or histopathology.	Rowe et al., 1952

A dose-related depression in mean body weight gain was observed in the male rats (8, 27 and 42% lower than control at 3, 15 and 75 ppm, respectively), but weight gain was depressed only at 75 ppm (36%) in the females (NTP, 1982). Mortality did not occur in any of the rats at any of the concentrations tested. Histopathological effects were found only in rats exposed to 75 ppm and included swelling and/or vacuolation of the adrenal cortical cells of the zona fasciculata, slight decrease in follicular size in the thyroid, and nasal cavity alterations (e.g., cytomegaly, focal hyperplasia, squamous metaplasia and loss of cilia).

A dose-related depression in mean weight gain was observed for male mice (3, 21 and 36% lower than control at 3, 15 and 75 ppm, respectively) (NTP, 1982). In male mice, 4/10 exposed to 3 ppm and 1/10 female mice exposed to 75 ppm died. Eye irritation was evident in mice exposed to 75 ppm during weeks 12 and 13, but histopathologic alterations were limited to the nasal cavity; effects that included cytomegaly, focal hyperplasia, squamous metaplasia and loss of cilia each occurred in 2-3 of the 10 males and 8-9 of the 10 females.

The NIOSH criteria document for EDB (NIOSH, 1977) cites an unpublished 13-week inhalation study that was conducted with $B6C3F_1$ mice (Ter Haar, 1977). The experimental design and results indicate that this study is the same as the NTP subchronic inhalation study (Reznik et al., 1980; NTP, 1982).

The results of a 13-week inhalation study that was conducted by the Dow Chemical Company are summarized in U.S. EPA (Office of Pesticide Programs) EDB Position Document 2/3 (U.S. EPA, 1980; Nitschkel, 1980). The dose levels were somewhat ambiguously reported in this summary, but it appears that male and female CDF (F344) rats were exposed to 0, 23, 77 or 3076 mg/m³ (0, 3, 10 or 400 ppm, respectively) EDB 6 hours/day, 5 days/week for 13 weeks for a total of 67-68 exposures in 95-96 days. The number of rats tested per dose was not reported, but scheduled sacrifices occurred after 1, 6 and 13 weeks of exposure. Toxicity evaluations apparently were based on body weight measurements and pathological examinations, but the extent of the examinations was not indicated in the summary. Rats exposed to 400 ppm EDB reportedly showed a definite adverse response as characterized by decreased body weight gain, increased liver and kidney weights, and histological alterations in the nasal turbinates that ranged from very slight epithelial hyperplasia to hyperplasia and nonkeratinizing squamous metaplasia. Slight epithelial hyperplasia of the nasal turbinates was found in rats that were exposed to 10 ppm EDB and necropsied after 1, 6 or 13 weeks, but these alterations were not present 88 days after the last exposure. Rats exposed to 3 ppm EDB reportedly showed no consistent effect in any parameter (not elaborated). It should be noted that mortality data were not provided and that the acute inhalation data of Rowe et al. (1952) (see Inhalation Exposure Section, Table V-2) indicate that 7-hour exposures to 400 ppm EDB would probably be lethal for rats. It is anticipated that a copy of this report will be available for detailed review and inclusion in a subsequent draft of this document.

Rowe et al. (1952) exposed groups of 20 rats of each sex, 8 guinea pigs of each sex, and 4 rabbits of both sexes (3 males, 1 female) 7 hours/day to 25 ppm EDB for 145-152 exposures in 205-214 days and 50 ppm EDB for 57-63 exposures in 80-91 days (Table V-5). The exposures were typically 5 days/week and unexposed and air-exposed matched animals were used as controls.

Body weights were determined twice weekly and pathological examinations were conducted on all animals; the lungs, heart, liver, spleen and testes were weighed, and tissues from these organs as well as the pancreas and adrenal glands were examined histologically. Other indices of toxicity occasionally were examined as indicated in Table V-5, but blood urea nitrogen and total liver lipid was determined in many of the animals. The results of these experiments indicate that subchronic exposure to 50 ppm EDB was not well tolerated by the rats or the guinea pigs; mortality and organ weight changes (increased liver, kidney, lung or spleen weight, decreased testis weight) occurred in the rats, and decreased growth, increased organ weights (lung, liver and kidney) and histological alterations (slight degenerative changes in the liver and kidney) occurred in the guinea pigs. A slight increase in liver and kidney weights was observed in the rabbits exposed to 50 ppm EDB, but other effects of exposure were not described. Adverse effects were not apparent in the guinea pigs or rabbits that were exposed to 25 ppm. There was also no evidence of systemic toxicity in the 25 ppm rats, but mortality occurred that was attributed to local respiratory effects (pneumonia and infection). Similar effects were described in groups of two monkeys (1 of each sex) that were exposed to 50 ppm (49 seven-hour exposures in 70 days) and 25 ppm (156 exposures in 220 days) EDB (Rowe et al., 1952) (see Table V-5); effects on body weight gain, organ weights (liver and kidneys) and organ histology (fatty degeneration of liver) were apparent at 50 ppm, but adverse effects were not indicated by the same criteria used in the rodent studies at 25 ppm.

The results of a carcinogenicity bioassay conducted using a single exposure level provided additional information regarding the non-neoplastic effects of inhaled EDB (Wong et al., 1982). In this study, groups of 48 Sprague-Dawley rats of each sex were exposed to 0 or 20 ppm EDB (99% pure) for 7 hours/day, 5 days/week for 18 months. Body weights were determined throughout the study, food consumption was determined after the first 3 months, and hematological evaluations (RBC, hematocrit, hemoglobin, WBC) were performed after 10-12 months of exposure. Comprehensive histopathological examinations (with the exception of the nasal cavity) were conducted as detailed in the Carcinogenicity Section. Treated rats had high mortality and depressed body weight gain during months 15-18, but food consumption was not significantly lower in any of the treated rats and hematology indices were unchanged (see Table V-5). A significant increase in the incidence of tumors in the spleen, adrenals and mammary gland were found (Carcinogenicity Section), but non-neoplastic lesions were described only in the spleen (atrophy in 0/48 controls and 6/48 treated, $p<0.05$); treatment-related testicular atrophy was not observed. Concurrent administration of 0.05% disulfiram in the diet enhanced the toxicity/carcinogenicity of EDB. Increased toxicity was indicated by earlier and complete mortality (most died within 12 months, all by month 14), depressed body weight gain from month 1, adverse hematological effects (decreased RBC count, hematocrit, hemoglobin) and testicular atrophy (90% vs. 2 or 4% in disulfiram or EDB control groups, respectively). Higher incidences of tumors were also observed in the EDB/disulfiram group (Carcinogenicity Section).

NTP (1982) conducted a carcinogenicity bioassay with groups of 50 F344 rats and B6C3F$_1$ mice of each sex that were exposed to 0, 10 or 40 ppm EDB by inhalation, 6 hours/day, 5 days/week for 78-103 weeks (mice) and 88-103 weeks (rats) (see Table V-5). Mean body weight of the high dose rats and mice were lower than those of the corresponding untreated controls throughout the study, and these animals exhibited limb or body weakness during the second year of treatment. Survival was sufficiently shortened in dose groups (see Table V-5) to produce significant responses ($p<0.001$) in the Tarone test for positive dose-related trend in mortality for each species and sex, except male mice; the control and dosed male mice had poor survival because of an ascending suppurative urinary tract infection that was not treatment related. Comprehensive histological examinations were conducted on all of the animals. Principal non-neoplastic findings in the rats included epithelial hyperplasia, squamous metaplasia and suppurative inflammation in the respiratory system (the most prominent alterations), liver necrosis, toxic nephropathy, testicular degeneration and atrophy, adrenal cortex degeneration (females) and retinal atrophy (females). The respiratory system was the target organ for non-neoplastic lesions in the mice (epithelial hyperplasia and inflammation in the nasal cavity, bronchus or lung). The non-neoplastic alterations in the respiratory system are consistent with the induction of high incidences of nasal cavity and lung tumors in the same animals (Carcinogenicity Section).

Target Organ Toxicity

EDB is absorbed readily by all routes of exposure and becomes widely distributed in mammalian tissues. Localization of EDB and/or its metabolites in particular organs (e.g., liver, kidneys) is in general agreement with reported sites of primary tissue damage. Since metabolism by microsomal oxidation and/or glutathione conjugation are proposed as an enhancer for expression of toxic effects of the parent compound (see Chapter III), some target organs for EDB would be expected to be metabolically active. Insofar as conjugation with glutathione is involved in the bioactivation of EDB to an ultimate mutagen/carcinogen, tissue susceptibility to preneoplastic/neoplastic effects should be influenced by the availability of glutathione and the activity of the glutathione-S-transferase enzymes.

The acute and subchronic/chronic animal studies that are summarized in the General Toxicity Section indicate that the liver (e.g., cloudy swelling, centrilobular fatty degeneration and necrosis), kidney (congestion, edema, toxic nephropathy) and testis (atrophy) are primary systemic targets of EDB toxicity regardless of route (oral or inhalation) or duration of exposure (Rowe et al., 1952; Nachtomi et al., 1968; Nachtomi and Alumot, 1972; Nachtomi and Sarma, 1977; Nachtomi and Farber, 1978; Nachtomi, 1980; NCI, 1978; NTP, 1982). Non-neoplastic effects also have been described in the adrenal cortex (swelling and vacuolation, degeneration) and spleen (atrophy) in some of the longer duration studies (Rowe et al., 1952; NCI, 1978; NTP, 1982; Wong et al., 1982). The most pronounced alterations in the longer duration studies were due to direct local effects of exposure; high incidences of lesions, some of which were preneoplastic, were induced in the forestomach (hyperkeratosis, acanthosis) in gavage studies and nasal cavity/respiratory system (inflammation, epithelial hyperplasia, squamous metaplasia) in inhalation studies (NCI, 1978; NTP, 1982; Stinson et al., 1981).

Symptoms consistent with CNS dysfunction (e.g., depression, agitation, restlessness, tremors, rapid respiration, unconsciousness) have been described in various animal studies, particularly at the higher concentrations in the inhalation studies (see General Toxicity Section). Although these observations indicate that EDB can adversely affect the CNS, the nature of these effects has not been further elucidated.

Other Effects

Carcinogenicity. One carcinogenicity bioassay has been conducted in which EDB was administered by gavage (NCI, 1978). In this study, technical grade EDB (purity >99%) was administered by gavage in corn oil to groups of 50 Osborne-Mendel rats and $B6C3F_1$ mice of each sex, at TWA dosages of 27.4 and 29.1 mg/kg/day (male rats), 26.7 and 28.1 mg/kg/day (female rats), and 43.9 and 76.6 mg/kg/day (male and female mice). Groups of 20 rats and mice of each sex served as vehicle controls. The dosing schedules and durations of treatment are detailed in Table V-6, but it should be noted that the exposures were 5 consecutive days/week, that the initial low and high dosages for the rats were 40 and 80 mg/kg/day, respectively, and that the initial low and high dosages for the mice were 60 and 120 mg/kg/day, respectively. High mortality and early onset of cancer prompted discontinuation of dosing from weeks 17-30 in the high dose rats (with resumption of dosing at the low dose level and subsequent treatment-free intervals as detailed in Table V-6), periodic adjustment of dose in the male and female mice (see Table V-6), and early termination of both rat and mouse studies (originally designed to last 110 and 90 weeks, respectively). There were no untreated observation periods for the treated rats, but the treated mice were observed for 24-37 weeks as detailed in Table V-6. Treatment-related mortality

TABLE V-6

Carcinogenesis Bioassays of EDB

Route (vehicle)	Species/ Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Target Organ, Tumor Type	Tumor Incidence^{b}	Reference
Gavage (corn oil)	rat, Osborne-Mendel	M	0 (vehicle control), 27.4 or 29.1 mg/kg/day^{a}	49 weeks	63 weeks (control) 49 weeks (treated)	stomach, squamous cell carcinoma	0/20^{c}, 45/50^{d}, 33/50^{c}	NCI, 1978
						circulatory system, hemangiosarcoma	0/20, 11/50^{d}, 4/50 (0/20, 11/50^{d}, 4/27)^{e}	
						thyroid, follicular cell adenoma/carcinoma	0/20^{h}, 5/50, 8/49 (0/20^{h}, 5/50, 8/48;^{e}	
		F	0 (vehicle control), 26.7 or 28.1 mg/kg/day^{f}	61 weeks	63 weeks (control) 61 weeks (treated)	stomach, squamous cell carcinoma	0/20^{c}, 40/50^{c}, 29/50^{c}	
						liver, hepatocellular carcinoma	(0/20^{h}, 1/35, 5/25^{h})^{e}	
						liver, carcinoma or nodule^{i}	(0/20^{d}, 1/35, 6/25^{d})^{e}	
Gavage (corn oil)	mouse, B6C3F1	M	0 (vehicle control), 43.9 or 76.6 mg/kg/day^{j}	53 weeks	59 weeks (control) 78 weeks (low dose) 77 weeks (high dose)	stomach, squamous cell carcinoma	0/20^{j}, 45/50^{c}, 29/49^{c} (0/19^{c}, 29/31^{c}, 24/34^{c})^{e}	NCI, 1978
						stomach, squamous cell papilloma/carcinoma	0/20^{c}, 45/50^{c}, 31/49^{c}	
						lung, alveolar/bronchiolar adenoma	0/20^{k}, 4/45, 10/47^{h} (0/19^{k}, 1/30, 8/34^{d})^{e}	
		F	0 (vehicle control), 43.9 or 76.6 mg/kg/day^{j}	53 weeks	60 weeks (control) 90 weeks (low dose) 78 weeks (high dose)	stomach, squamous cell carcinoma	0/20^{d}, 46/49^{c}, 28/50^{c} (0/19^{c}, 9/10^{c}, 11/14^{c})^{e}	
						lung, alveolar/bronchiolar adenoma	0/20, 10/43^{d}, 6/46 (0/19^{d}, 2/10, 4/14^{g})^{e}	
						lung, alveolar/bronchiolar adenoma/carcinoma	0/20, 11/43^{k}, 6/46	

TABLE V-6 (cont.)

Route (vehicle)	Species/ Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Target Organ, Tumor Type	Tumor Incidence[b]	Reference
Inhalation (vapor)	rat, F344	M	0 (chamber control), 10 or 40 ppm, 6 hours/day, 5 days/week[l]	0 weeks (control), 103 weeks (low dose), 88 weeks (high dose)	104-106 weeks (control), 104 weeks (low dose), 88-89 weeks (high dose)	nasal cavity, adenoma	0/50, 11/50[c], 0/50	NTP, 1982[n]
						nasal cavity, carcinoma	0/50[c], 0/50, 21/50[c]	
						nasal cavity, adenocarcinoma	0/50[c], 20/50[c], 28/50[c]	
						nasal cavity, tumors[m]	0/50[c], 39/50[c], 41/50[c]	
						circulatory system, hemangiosarcoma	0/50[c], 1/50, 15/50[c]	
						pituitary, adenoma	0/45, 7/48[k], 2/47	
						tunica vaginalis, mesothelioma	0/50[c], 7/50[k], 25/50[c]	
						tunica vaginalis, mesothelioma or malignant	1/50[c], 8/50[d], 25/50[c]	
		F	0 (chamber control), 10 or 40 ppm, 6 hours/day, 5 days/week[l]	0 weeks (control), 103 weeks (low dose), 91 weeks (high dose)	104-106 weeks (control), 104 weeks (low dose), 91-92 weeks (high dose)	nasal cavity, adenoma	0/50, 11/50[c], 3/50	
						nasal cavity, carcinoma	0/50[c], 0/50, 25/50[c]	
						nasal cavity, adenocarcinoma	0/50[c], 20/50[c], 29/50[c]	
						nasal cavity, tumors[m]	1/50[c], 34/50[c], 43/50[c]	
						lung, alveolar/bronchiolar carcinoma/adenoma	0/50[c], 0/48, 5/47[g]	
						circulatory system, hemangiosarcoma	0/50[k], 0/50, 5/50[g]	
						pituitary, adenoma	1/50, 18/49[c], 4/45	
						mammary gland, fibroadenoma	4/50[k], 29/50[c], 24/50[c]	
Inhalation (vapor)	mouse, B6C3F$_1$	M	0 (chamber control), 10 or 40 ppm, 6 hours/day, 5 days/week[l]	0 weeks (control) 78 weeks (treated)	79 weeks (control) 78-79 weeks (treated)	lung, alveolar/bronchiolar carcinoma	0/41[c], 3/48, 19/46[c]	NTP, 1982[n]
						lung, alveolar/bronchiolar adenoma/carcinoma	0/41[c], 3/48, 23/46[c]	
						respiratory system, tumors[o]	0/41[c], 3/48, 25/46[c]	

TABLE V-6 (cont.)

Route (vehicle)	Species/ Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Target Organ, Tumor Type	Tumor Incidence[b]	Reference
Inhalation (vapor)		F	0 (chamber control), 10 or 40 ppm, 6 hours/day, 5 days/week[l]	0 weeks (control) 103 weeks (low dose) 90 weeks (high dose)	104-106 weeks (control) 104 weeks (low dose) 90-91 weeks (high dose)	nasal cavity, carcinoma	0/50[c], 0/50, 6/50[d]	NTP, 1982[n]
						nasal cavity, tumors[p]	0/50[c], 0/50, 12/50[c]	
						lung, alveolar/bronchiolar carcinoma	1/49[c], 5/49, 37/50[c]	
						lung, alveolar/bronchiolar adenoma/carcinoma	4/49[c], 11/49[h], 41/50[c]	
						respiratory system, tumors[o]	4/49[c], 11/49[h], 41/50[c]	
						subcutaneous tissue or rib, fibrosarcoma	0/50[c], 5/50[g], 11/50[c]	
						circulatory system, hemangioma or hemangiosarcoma	0/50[c], 11/50[c], 23/50[c]	
						mammary gland, adenocarcinoma	2/50, 14/50[c], 8/50[h]	
Inhalation (vapor)	mouse, B6C3F1	M	0 (chamber control), 10 or 40 ppm, 6 hours/day, 5 days/week[l]	0 weeks (control) 103 weeks (low dose) 90 weeks (high dose)	104 weeks (control) 104 weeks (low dose) 91 weeks (high dose)	nasal cavity, focal epithelial hyperplasia	0/45, 1/44, 10/46	Stinson et al., 1981
						nasal cavity, squamous papilloma	0/45, 0/44, 3/46	
						nasal cavity, carcinoma[q]	0/45, 0/44, 0/46	
						nasal cavity, hemangiosarcoma	0/45, 0/44, 0/46	
Inhalation (vapor)	mouse, B6C3F1	F	0 (chamber control), 10 or 40 ppm, 6 hours/day, 5 days/week[l]	0 weeks (control) 103 weeks (low dose) 90 weeks (high dose)	104 weeks (control) 104 weeks (low dose) 91 weeks (high dose)	nasal cavity, focal epithelial hyperplasia	0/50, 3/49, 11/49	Stinson et al., 1981
						nasal cavity, squamous papilloma	0/50, 0/49, 7/49	
						nasal cavity, carcinoma[q]	0/50, 0/49, 7/49[r]	
						nasal cavity, hemangiosarcoma	0/50, 0/49, 2/49	
						nasal cavity, sarcoma poorly differentiated	0/50, 1/49, 0/49	

TABLE V-6 (cont.)

Route (vehicle)	Species/ Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Target Organ, Tumor Type	Tumor Incidence[b]	Reference
Inhalation (vapor)	rat, Sprague-Dawley	M	0 or 20 ppm, 7 hours/day, 5 days/week[s]	18 months (72 weeks)	18 months (72 weeks)	spleen, hemangiosarcoma	0/48, 6/46[h]	Wong et al., 1982
						adrenal tumors[t]	2/48, 11/46[h]	
						subcutaneous, mesenchymal tumor	3/48, 11/46[h]	
						total tumors	8/48, 25/46[h]	
		F	0 or 20 ppm, 7 hours/day, 5 days/week[s]	18 months (72 weeks)	18 months (72 weeks)	spleen, hemangiosarcoma	0/48, 6/45[h]	
						adrenal tumors[t]	1/48, 6/45[h]	
						mammary gland, tumors[u]	2/48, 25/45[h]	
						total tumors	8/48, 29/45[h]	
Dermal (acetone)	mouse, Ha:ICR Swiss	F	0 (vehicle control), 25 or 50 mg (~0, 833 or 1667 mg/kg, assuming 0.03 kg bw)	3 times/ week for 440-594 days[v]	440-594 days[v]	skin, papilloma	0/30, 2/30, 8/30[c]	Van Duuren et al., 1979
						skin, carcinoma	0/30, 2/30, 3/30	
						lung, papilloma	11/30, 24/30[k], 26/30[c]	
						lung, carcinoma	0/30, 0/30, 0/30	
						forestomach, papilloma	2/30, 3/30, 3/30	
						forestomach, carcinoma	0/30, 1/30, 0/30	
Dermal (acetone)	mouse, Ha:ICR Swiss	F	2.5 μg PMA (positive control), 5 μg PMA (positive control) or 75 mg EDB (single dose) followed by 5 μg PMA[w]	3 times/ week for 376 days (controls) EDB followed by PMA 3 times/ week for 428-576 days	376 days[x] (controls) 428-576 days[x] (treated)	skin, papilloma	9/120, 6/90, 2/30	Van Duuren et al., 1979
						skin, carcinoma	10/120, 7/90, 0/30	

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[a] TWA dose. Groups of 20 control and 50 treated males were exposed 5 consecutive days/week with technical grade (>99% pure) EDB. Low dose: 40 mg/kg x 41 weeks, 0 mg/kg x 1 week, 40 mg/kg x 4 weeks, 0 mg/kg x 1 week and 40 mg/kg x 2 weeks. High dose: 80 mg/kg x 16 weeks, 0 mg/kg x 13 weeks, 40 mg/kg x 12 weeks, 0 mg/kg x 1 week, 40 mg/kg x 4 weeks, 0 mg/kg x 1 week and 40 mg/kg x 2 weeks.

[b] Tumor incidences listed for control group to high dose group. For the NCI/NTP bioassay, the probability levels for the Cochran-Armitage and Fischer exact tests are given beneath the incidence of tumors in the control and treated groups, respectively, when $p<0.05$. The data were not statistically analyzed in the Stinson et al. (1981) study. The statistical test used in the Wong et al. (1982) study was not specified, and Chi-square analysis was used in the Van Duuren et al. (1979) study.

TABLE V-6 (cont.)

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[c] $p<0.001$

[d] $p<0.02$

[e] Time-adjusted analyses (due to high early mortality) included those animals that survived at least until the time of first tumor appearance (rats, mice) or at least 52 weeks (mice), which ever occurred earlier.

[f] TWA dose. Groups of 20 control and 50 treated females were exposed 5 consecutive days/week with technical grade (>99% pure) EDB. Low dose: 40 mg/kg x 41 weeks, 0 mg/kg x 1 week, 40 mg/kg x 4 weeks, 0 mg/kg x 1 week, 40 mg/kg x 4 weeks, 0 mg/kg x 1 week, 40 mg/kg x 4 weeks, 0 mg/kg x 1 week and 40 mg/kg x 4 weeks. High dose: 80 mg/kg x 16 weeks, 0 mg/kg x 13 weeks, 40 mg/kg x 12 weeks, 0 mg/kg x 1 week, 40 mg/kg x 4 weeks, 0 mg/kg x 1 week, 40 mg/kg x 4 weeks, 0 mg/kg x 1 week, 40 mg/kg x 4 weeks, 0 mg/kg x 1 week and 40 mg/kg x 4 weeks.

[g] $p<0.03$

[h] $p<0.05$

[i] Hepatocellular carcinoma or neoplastic nodule

[j] TWA dose. Treatment for 5 consecutive days/week with technical grade (>99% pure) EDB. Low dose: 60 mg/kg x 10 weeks, 100 mg/kg x 2 weeks and 60 mg/kg x 41 weeks. High dose: 120 mg/kg x 10 weeks, 200 mg/kg x 2 weeks, 120 mg/kg x 27 weeks and 60 mg/kg x 14 weeks.

[k] $p<0.01$

[l] Groups of 50 control and treated animals were exposed to 99.3% pure EDB.

[m] Includes adenoma, adenocarcinoma, adenomatous polyp, squamous cell carcinoma, papillary adenoma, squamous cell papilloma and/or carcinoma

[n] Incidences of the following tumors showed a positive dose-related trend ($p<0.05$ in Cochran-Armitage test), but were not significantly elevated in either dose group when compared with the control group ($p>0.05$ in Fischer exact test): salivary gland sarcoma (male rats), thyroid follicular-cell adenoma or carcinoma (male rats), subcutaneous fibroma (female rats), subcutaneous fibroma or fibrosarcoma (female rats), liver hepatocellular carcinoma (female rats), mammary gland adenocarcinoma (female rats) and circulatory system hemangioma or hemangiosarcoma (male mice).

[o] Tumors of the lung, bronchus and bronchiole including adenoma, carcinoma, adenomatous polyp, alveolar/bronchiolar adenoma and/or alveolar/bronchiolar carcinoma.

[p] Includes adenoma, carcinoma, adenomatous polyp and hemangiosarcoma

[q] Includes squamous carcinoma, adenocarcinoma and mixed carcinoma

[r] Includes 2 squamous carcinoma, 2 adenocarcinoma and 3 mixed carcinoma

[s] Groups of 48 rats were exposed to 99% pure EDB

[t] Includes phenochromocytoma, cortical adenoma or carcinoma

[u] Includes adenoma, fibroadenoma, carcinoma, adenocarcinoma

[v] Range reported for nine compounds; duration of the EDB study was not specifically reported.

[w] EDB was administered to dorsal skin once, followed 14 days later by PMA (phorbol myristate acetate) 3 times weekly. Treatments administered in 0.2 ml acetone.

[x] Median survival time of the PMA-treated controls was 376 days. Median survival time of 15 halohydrocarbon-treated mice (including EDB-treated mice) was 428-576 days.

occurred in the rats, and all surviving dosed males and females were sacrificed after 49 and 61 weeks of EDB administration (Oral Exposure Section).

Squamous cell carcinomas of the forestomach appeared as early as 12-15 weeks. These tumors developed in 97-100% of the high dose males, low and high dose females that survived beyond week 15. Initially the tumors invaded locally and eventually metastasized throughout the abdominal cavity. The overall incidences observed were 45/50, 33/50, 40/50 and 29/50 in the low dose males, high dose males, low dose females and high dose females, respectively. No tumors of this type were observed in control rats. In addition, hyperkeratosis and acanthosis, considered to be part of the spectrum of chemically induced gastric lesions (NCI, 1978), were present in many of the high dose rats (Oral Exposure Section). Statistically significant ($p<0.02$) increased incidences of hemangiosarcomas were found in the low dose male rats, but the Cochran-Armitage test did not show a positive association between dosage and occurrence of this tumor (see Table V-6). Treated male rats also showed an increased incidence of thyroid tumors (follicular cell adenomas or carcinomas), but only the Cochran-Armitage test was significant ($p=0.04$). The only other tumor type that occurred in the female rats at a statistically significant incidence was hepatocellular carcinoma; when time-adjusted incidences were used (see Table V-6), the Cochran-Armitage test showed a positive association with dose ($p<0.02$) that was supported by a significant Fisher exact test comparison ($p=0.02$) of high dose to vehicle control.

In the mouse, there were early deaths that were treatment-related (NCI, 1978) (see Oral Exposure Section). Squamous cell carcinomas were observed in the forestomach, which could have been the cause of death along with the systemic toxicity of EDB. All surviving mice were sacrificed by week 78 (all males and high dose females) or 37 weeks after 53 weeks of treatment (low dose females). The cumulative forestomach carcinomas were scored: 45/50, 29/49, 46/49 and 28/50 of the low dose males, high dose males, low dose females and high dose females, respectively; none were observed in the vehicle control groups. Forestomach carcinomas developed in 78-92% of the male mice that survived 26 weeks and in 76-100% of the female mice that survived 14-18 weeks of treatment and, as with the rats, eventually metastasized to the peritoneal cavity and lungs. Significantly increased incidences of primary lung tumors (alveolar/bronchiolar adenomas) also occurred in the treated mice of both sexes but in none of the controls (see Table V-6). Although testicular atrophy was found in the high dose males (Oral Exposure Section), increased incidences of neoplasms in this tissue were not reported.

A carcinogenesis bioassay by inhalation was conducted by exposing groups of 50 F344 rats and B6C3F$_1$ mice of each sex to 0, 10 or 40 ppm EDB (99.3-99.4% pure) for 78-106 weeks as detailed in Table V-6 (NTP, 1982). The animals were sacrificed within 1 week of the end of treatment and comprehensive pathological examinations were conducted on all animals.

Mean body weight was lower and survival was shorter in the high dose rats of both sexes than in the corresponding unexposed (chamber) controls (NTP, 1982) (see previous Exposure Section). The incidences of tumors of the nasal cavity, particularly malignant carcinomas and adenocarcinomas, were increased significantly in the treated rats of both sexes (see Table V-6); for adenocarcinomas or total tumors, significantly higher incidences in both dose groups ($p<0.001$, Fisher exact test) and positive dose-related trends ($p<0.001$, Cochran-Armitage test) occurred. Adenomas were increased significantly ($p<0.001$) in the low dose rats of either sex. In addition, the combined incidence of alveolar/bronchiolar adenomas and carcinomas of the lung was statistically significant ($p<0.03$) for the high dose females and showed a positive association with dose ($p<0.001$, Cochran-Armitage test). Tumors at other sites included hemangiosarcomas (particularly in the spleen) that were significantly higher in the high dose groups of both sexes ($p<0.001$, males and $p<0.03$, females), and occurred with positive linear trends ($p<0.001$, males and $p=0.002$, females). The incidences of mesotheliomas in the tunica vaginalis in treated males ($p<0.01$) and mammary gland fibroadenomas in treated females ($p<0.001$) were higher than in the respective controls, and occurred with significant dose-related trends (see Table V-6). Statistically increased incidences of pituitary adenomas also occurred in the low dose rats of both sexes ($p<0.01$, males and $p<0.001$, females), but the incidences of this tumor were not significantly increased at the high doses and the Cochran-Armitage test for positive linear trend was not significant.

Mean body weight of the high dose mice of either sex were lower and survival in the low- and high-dose female mice was shorter than in the corresponding controls (NTP, 1982) (see Inhalation Studies Section). Poor survival also occurred in the control and treated male mice, but the principal cause of death was nontreatment-related urinary tract infection.

As with the rats, high incidences of tumors were induced in the nasal cavity and/or respiratory system of the mice (see Table V-6). Alveolar/bronchiolar carcinoma occurred at incidences that were elevated significantly ($p<0.001$, Fisher exact test) in the high dose groups of both sexes and positively associated with dose ($p<0.001$, Cochran-Armitage test). Nasal cavity tumors, particularly carcinoma, also occurred at significant incidences ($p<0.001$) in the high dose females, but were not increased in the males. Hemangiosarcomas of the circulatory system, subcutaneous fibrosarcomas and mammary gland adenocarcinomas also occurred at significantly elevated incidences in the low and high dose female mice when compared with the unexposed controls (see Table V-6); the Cochran-Armitage test indicated significant positive dose-related trends in the incidences of females with the hemangiosarcomas and fibrosarcomas.

Proliferative lesions in the nasal cavities of B6C3F$_1$ mice were characterized in detail in another study that was identical in experimental design to the NTP (1982) inhalation carcinogenesis bioassay (Stinson et al., 1981). Groups of 50 male and female mice were exposed to 0, 10 or 40 ppm EDB 6 hours/day, 5 days/week for 104 weeks (unexposed chamber controls and low dose mice) or 91 weeks (high dose mice). Survivors in the dosed groups were sacrificed 1 week following termination of exposure and control animals were sacrificed after 104 weeks. Gross examinations were reportedly conducted on all major tisssues, organs and lesions, but the nasal cavity was the only site that was examined histologically and gross pathological effects at other sites were not reported. Body weight and survival data were not reported.

The results of this study (see Table V-6) suggest that the occurrence of epithelial hyperplastic lesions was related to dose in both the males and females. Incidences of benign squamous papillomas also appeared to be somewhat elevated in the high dose mice of each sex (3/46 males, 7/49 females, none in the low dose or control groups), and the incidence of carcinoma was elevated in the high dose females (0/50, 0/49 and 7/49 in the control, low dose and high dose groups, respectively). The statistical significance of the above data was not determined, but it should be noted that the incidence of nasal cavity carcinoma is quite consistent with the significant data that were reported in the NTP (1982) bioassay (see Table V-6). Hemangiosarcomas were found in the nasal cavities of 2/49 high dose female mice, but hemangiosarcomas were not observed at this site in the NTP bioassay.

A chronic study was conducted with Sprague-Dawley rats to assess the carcinogenicity of inhaled EDB and the effect of dietary disulfiram (a blocking agent for aldehyde dehydrogenase on cytochrome P-450) on the carcinogenicity of EDB (Wong et al., 1982). This study was sponsored by NIOSH, conducted at the Midwest Research Institute, and had groups of 48 rats of each sex that were exposed to 0 or 20 ppm EDB (reported to be 99% pure) for 7 hours/day, 5 days/week for 18 months. Comprehensive histopathological examinations were conducted on all animals [except for 2 treated males and 3 treated females which was (due to autolysis)], but the nasal cavity was not examined.

The EDB-exposed rats had treatment-related high mortality and depressed body weight gain as detailed in Subchronic and Chronic Toxicity Section.

Significantly ($p<0.05$) increased incidences of tumors were found in the spleen (hemangiosarcoma) and adrenal gland (pheochromocytoma, cortical adenoma or carcinoma) in both the treated males and females, and an increased ($p<0.05$) incidence of mammary tumors (adenoma, fibroadenoma, carcinoma or adenocarcinoma) also was found in the females (see Table V-6). Lung tumors (hemangiosarcoma or bronchogenic carcinoma) were noted in 3/46 treated males and in none of the control males, but this incidence was not statistically significant ($p>0.05$). The nasal cavity was not, as mentioned previously, examined in this study. Concurrent administration of 0.05% disulfiram in the diet caused a substantial increase ($p<0.05$) in the incidence of tumors in the liver (hepatocellular tumor), kidney (adenoma and adenocarcinoma), thyroid (follicular epithelial adenoma) and mesentary or omentum (hemangiosarcoma) in both the male and female rats as compared with rats receiving either EDB or disulfiram alone. Male rats that received combined EDB and disulfiram also had a significantly higher ($p<0.05$) incidence of lung tumors than EDB-treated rats or the unexposed or disulfiram controls.

Van Duuren et al. (1979) conducted a lifetime dermal application study in which EDB in acetone was applied 3 times weekly to the skin of female Ha:ICR Swiss mice at doses of 0 (vehicle control), 25 or 50 mg/animal (~0, 833 or 1667 mg/kg, assuming a body weight of 0.03 kg). In this test, EDB was assayed as a complete carcinogen, i.e., initiator and promotor. Thirty mice per group were tested, the EDB was applied to shaved dorsal skin, and the high dose represented a maximum tolerated dose as determined in a preliminary experiment. Histopathologic examinations were conducted on the skin, liver, stomach, kidneys and all abnormal-appearing tissues and organs.

The results of this bioassay showed that incidence of local skin papillomas and distant lung papillomas were significantly ($p<0.05$) increased from control levels at the high dose, and that lung papillomas were significantly ($p<0.05$) increased at the low dose (see Table V-6). Mice were housed six to a cage with no restraining collars to prevent licking during grooming. Thus aspiration to the lungs could have occurred during grooming. Increased incidences of carcinomas in the skin or lungs, or papillomas or carcinomas in the forestomach were not found at either dose. Initiation with EDB (a single 75 mg dermal application to 30 female mice), followed 14 days later by promotion with phorbol myristate acetate (3 times/week at 5 μg/mouse for a lifetime), did not elicit a significant increase in the incidence of local papillomas or carcinomas (see Table V-6).

Mutagenicity.

Mutation Assays in Microorganisms and Cultured Cells -- In the initial report by Ames and Yanofsky (1971) describing the use of specially constructed histidine-dependent strains of *Salmonella typhimurium* to detect chemical mutagens, EDB was reported to be mutagenic in strains his G46 and TA1530 at exposure levels of 5 μℓ/plate. Following this initial study, EDB has been included in a number of validation studies of the *S. typhimurium* assay system and in the studies surveying commercially and environmentally important chemicals for mutagenic activity. In a test of 300 chemicals for mutagenic activity conducted by the Ames group (McCann et al., 1975) to validate the empirical association between mutagenic and carcinogenic activity of chemicals, EDB was considered weakly mutagenic in strains TA100 and TA1535, while negative responses were obtained with the other strains tested, TA1537, TA98 and TA1538. The reversion rate was ~0.06

colonies/nmole in the most sensitive strain, TA100. These initial assays were performed without the benefit of a mammalian metabolic activation system (S-9).

Moriya et al. (1983) included EDB in a survey of 228 pesticides for mutagenic activity. At dose levels of between 50 and 5000 µg/plate, EDB produced a dose-dependent increase in histidine-independent colonies in strain TA100 (>10-fold at 5000 µg/plate). Identical responses were observed regardless of whether an S-9 activation system was included in the assay. Although quantitative data were not presented, it was reported that positive responses also were observed with strains TA1535 and TA98 (weak), while strains TA1538 and TA1537 produced negative results. Principe et al. (1981) conducted a small survey on the mutagenic activity of industrial and agricultural chemicals. At a dose of 100 µℓ/plate, EDB increased the number of revertant colonies in strains TA1535, TA100 and TA98, but not TA1537 or TA1538. Addition of S-9 in this study decreased the mutagenic response in all positive strains. In a survey of the mutagenicity of 2- and 3-carbon halogenated compounds performed by Stolzenberg and Hine (1980), EDB again produced a positive mutagenic response in strain TA100. At the doses tested, 0.1, 1 and 10 µmoles/plate (18.7, 187 and 1870 µg/plate), 25, 353 and 1530 revertants/plate were counted when the assay was performed with S-9, while similar results were observed at the two lower doses and complete cell death (absence of lawn) at the highest dose when the assay was performed without S-9. The results of these assays and other mutagenicity assays are summarized in Table V-7.

TABLE V-7

Mutagenicity Assays of EDB in Microorganisms and Cultured Cells

Assay	Indicator Organism	Strain	Concentration/Dose	Application Methods	S-9 Activation System[a]	Response[b]	Comments	Reference
Reverse mutation	*S. typhimurium*	G46 TA1530	5 µl 5 µl	plate incorporation	- -	+ +	Data were presented qualitatively as photographs of the plates.	Ames and Yanofsky, 1971
Reverse mutation	*S. typhimurium*	TA100 TA1535 TA1537 TA98 TA1538	NR NR NR NR NR	plate incorporation	- - - - -	+ + - - -	Quantitative data were provided only for strain TA100 in which 0.06 revertant/nmol were produced.	McCann et al., 1975
Reverse mutation	*S. typhimurium*	TA100 TA1535 TA98 TA1537 TA1538	50-5000 µg/plate 50-5000 µg/plate 50-5000 µg/plate 50-5000 µg/plate 50-5000 µg/plate	plate incorporation	+/- +/- +/- +/- +/-	+/+ +/+ +/+ -/- -/-	Quantitative data were presented only for strain TA100 in which a greater than doubling of revertant colonies occurred at 500 µg/plate. The response in TA98 was described as weak.	Moriya et al., 1983
Reverse mutation	*S. typhimurium*	TA100	19-1900 µg/plate	plate incorporation	+/-	+/+	A greater than doubling of the spontaneous rate occurred at 190 µg/plate and the inclusion of S-9 protected the cell from toxicity at the highest dose.	Stolzenberg and Hine, 1980
Reverse mutation	*S. typhimurium*	TA1535	300 µg	spot test	+/-	+/-	Other doses may have been tested, but results were reported only for the 300 µg test.	Shiau et al., 1980
Reverse mutation	*S. typhimurium*	TA1535	~10-300 µg/plate	plate incorporation	+	-	There was a slight increase in the number of revertant colonies, but it was never above 2 times the background and was not dose-related.	Shiau et al., 1980
Reverse mutation	*S. typhimurium*	TA1530 TA1535 TA1538	244-1875 µg/plate 244-1875 µg/plate 244-1875 µg/plate	spot test	- - -	+ + -	The colonies were evenly distributed on the plate, indicative of the compound volatilizing and redistributing over the entire plate.	Rosenkranz, 1977; Brem et al., 1974

TABLE V-7 (cont.)

Assay	Indicator Organism	Strain	Concentration/Dose	Application Methods	S-9 Activation System^a	Response^b	Comments	Reference
Reverse mutation	*S. typhimurium*	TA98 TA100 TA1535 TA1537 TA1538	2644-20,962 μg/plate 2644-20,962 μg/plate 2644-20,962 μg/plate 2644-20,962 μg/plate 2644-20,962 μg/plate	vapor phase	+/- +/- +/- +/- +/-	+/+ +/+ +/+ -/- -/-	The concentration of compound tested was determined by GLC analyses of aqueous media in the test chamber. The lowest dose tested produced a >10-fold increase in revertant colonies for all positive strains.	Barber et al., 1981
Reverse mutation	*S. typhimurium*	TA1535 TA100 TA98 TA1537 TA1538	100 μℓ/plate 100 μℓ/plate 100 μℓ/plate 100 μℓ/plate 100 μℓ/plate	plate incorporation	+/- +/- +/- +/- +/-	+/+ +/+ +/+ -/- -/-	Metabolic activation decreased the mutagenic response.	Principe et al., 1981
Reverse mutation	*S. typhimurium*	G46	500 mg/kg s.c. injection	host-mediated NMRI mice	+	+	The increase in mutation frequency was significant at $p<1\%$.	Buselmaier et al., 1972, 1973
Reverse mutation	*S. typhimurium*	G46	NR	spot test	-	+	NC	Buselmaier et al., 1972, 1976
Reverse mutation	*S. marcescens*	a21	500 mg/kg s.c. injection	host-mediated NMRI mice	+	-	Leucine-independent colonies were counted.	Buselmaier et al., 1972, 1976
Reverse mutation	*E. coli*	WP2 uvrA	3750-1,875,000 μg/plate	plate incorporation	-	+	The range of doses tested was described in a general protocol for this study and may not be applicable for EDB.	Hemminki et al., 1980
Reverse mutation	*E. coli*	WP2 hcr	0-5000 μg/plate	plate incorporation	NR	+	Specific details on effective doses and whether response was observed in the presence or absence of S-9 were not provided.	Moriya et al., 1983
Reverse mutation	*B. subtilis*	TKJ5211	100 μg	spot test	+/-	+/-	NC	Shiau et al., 1980

TABLE V-7 (cont.)

Assay	Indicator Organism	Strain	Concentration/Dose	Application Methods	S-9 Activation System[a]	Response[b]	Comments	Reference
Forward mutation	*B. subtilis*	TKJ6321	100 µg	spot test	+/-	+/-	NC	Shiau et al., 1980
Recessive lethal	*N. crassa*	NR	1.6 µℓ/mℓ for 3 hours	liquid incubation	-	+	Study reported only in abstract form.	Malling, 1969
Forward mutation	*S. coelicolor*	NR	NR	spot test	-	+	Streptomycin resistant colonies were counted.	Principe et al., 1981
Forward mutation	*S. coelicolor*	NR	2-100 µℓ/plate	plate incorporation	-	-	Positive results were obtained with this organism in the spot test and hence a sufficiently high concentration may not have been tested.	Principe et al., 1981
Forward mutation	*A. nidulans*	35	NR	spot test	-	+	8-Azaguanine resistant colonies were counted.	Principe et al., 1981
Forward mutation	*A. nidulans*	35	10-200 µℓ/plate	plate incorporation	-	+	A doubling of mutant colonies was observed at 50 µℓ/plate.	Principe et al., 1981
Forward mutation	CHO/HGPRT	NA	9.4-562 µg/mℓ for 5 hours	liquid media	+/-	+/-	In the presence of S-9, activity was observed at the lowest dose tested, while in the absence of S-9, activity was not apparent at concentrations <47 µg/mℓ.	Tan and Hsie, 1981
Forward mutation	mouse lymphoma cells	L5178y	188-562 µg/mℓ for 2 hours	liquid media	+/-	+/+	Mutagenicity and toxicity were greater in the presence of S-9.	Clive et al., 1979

[a] "+" = with; "-" = without

[b] "+" = positive; "-" = negative

NA = Not applicable; NC = no comment; NR = not reported

Shiau et al. (1980) tested EDB for mutagenic activity in _S_. _typhimurium_ strain TA1535 using the spot test and observed no increase in revertant colonies in the absence of S-9, while a positive response was reported when 300 µg was applied to the spot in the presence of S-9. Further testing using doses of up to 300 µg/plate in the plate incorporation assay failed to demonstrate a mutagenic response. The volatile nature of EDB may have contributed to the lack of activity observed in this study as compared with the results of previously described assays. Rosenkranz (1977) and Brem et al. (1974) noted the problem of possible loss of test compound upon addition of volatile compounds to molten agar (45°C) during the preparation of plates for the plate incorporation assay. These investigators used what they described as a semiquantitative spot test in which various doses of EDB were applied to filter discs and the number of subsequently produced revertant colonies were counted. Using this technique, a dose-related increase in revertants was observed between 2.5 and 10 µmole/plate (244-1875 µg/plate) with strains TA1530 and TA1535. The assay was performed without S-9, and the frameshift sensitive strain, TA1538, was not mutated. Photographs of typical plates showed that the colonies were relatively evenly distributed over the entire plate (except for a small zone of inhibition around the disc), suggesting that the compound volatilized from the disc and was subsequently redeposited uniformly on the plate. With the use of this procedure, it was still unclear as to the extent of compound loss from the plate.

In order to eliminate problems associated with volatilization, Barber et al. (1981) used a closed system to expose test plates containing _S_. _typhimurium_ strains TA1535, TA1537, TA1538, TA98 and TA100 to the vapors of EDB.

It was demonstrated that distribution between the agar and vapor phase was complete in ~1 hour and that total recovery of the solvent after a 48-hour incubation was nearly 100%. The amount of EDB dissolved in the agar was determined by GLC to be 17.1, 28.2, 56.4 and 111.8 μmoles/plate (2644, 5287, 10,575 and 20,962 μg/plate) and at these concentrations a dose-related increase in revertant colonies was observed for all strains except strains TA1537 and TA1538. The inclusion of a mammalian metabolic activation system did not appreciably alter the results. For the most sensitive strain, TA1535, it was calculated that a vapor concentration of 1.7 ppm would be the minimum detectable level.

The *S. typhimurium*, as indicator organisms, also have been used in conjunction with *in vivo* metabolic activation. Buselmaier et al. (1972, 1976) injected NMRI mice subcutaneously with EDB at a dose of 500 mg/kg in a host-mediated assay. The subsequently reisolated strain G46 cells had a mutation frequency of 6.23×10^{-8} as compared with a frequency of 0.77×10^{-8} for cells from control mice. Similar positive results were obtained *in vitro* by these workers using the spot test. Employing the same host-mediated system with *Serratia marcescens* a21 as the indicator organism resulted in no increase in mutation frequency following EDB treatment.

Other prokaryotic microorganisms also have been used to evaluate the mutagenic potential of EDB. Hemminki et al. (1980) reported that EDB was mutagenic in *Escherichia coli* strain WP2 uvrA. Although specific dose information was not provided, the rate of formation of tryptophan-independent colonies was 78% of that expected for epichlorohydrin, which was used in this study as a standard mutagen for comparison. Using a similar system, Moriya et al. (1983) reported that EDB was mutagenic in *E. coli* strain WP2 hcr; however, the only experimental conditions described were in a general protocol, which indicated only that most chemicals were tested at doses of up to 5000 μg/plate and that the assay was performed both with and without the addition of S-9. In *B. subtilis* strains TKJ5211 and TKJ6321, which detect mutation to histidine-independence and forward mutations in the suppressor gene for histidine and methranine, respectively, EDB produced mutant colonies only in the presence of a mammalian metabolic activation system (Shiau et al., 1980). The compound was assayed by the spot test and gave a >2-fold increase in mutant colonies at a dose level of 100 μg.

In eukaryotic *in vitro* systems, EDB has been tested for mutagenicity in both fungi and cultured mammalian cells. In an abstract, Malling (1969) reported that EDB increased recessive lethal mutations in *Neurospora crassa*. At a dose level of 1.6 μg/mℓ, the mutation frequency for treated cells was 30/10^6 survivors as compared with 0.5/10^6 survivors for the controls. In the forward mutation assays measuring streptomycin resistant colonies of *Streptomyces coelicolor*, Principe et al. (1981) only detected a positive response using the spot test, while with the plate incorporation assay the response was negative. The highest dose tested in the plate incorporation assay was 100 μℓ/plate. In the same report, EDB increased 8-azaguanine resistant mutant colonies of *Aspergillus nidulans* haploid strain 35 in both the spot test and plate incorporation assay. In the plate incorporation assay a doubling of mutant colonies was detected at 50 μℓ/plate, with a 3.5-fold increase observed at 200 μℓ/plate, the highest dose tested. None of the above assays used a metabolic activation system.

Mutagenic tests in cultured mammalian cells have been conducted with both Chinese hamster ovary (CHO) cells and L5178Y lymphoma cells derived from mice. Tan and Hsie (1981) used the CHO/hypoxanthine-guanine phosphoribosyl transferase (HGPRT) system, which detects forward mutations to 6-thioguanine-resistant cells to evaluate the mutagenicity of EDB. At concentrations between 0.05 and 0.4 mM (9.4 and 75.0 μg/mℓ) and exposure periods of 5 hours, CHO cells had a dose-related increase in mutation frequency when S-9 was included in the assay. In the absence of S-9, a dose-related increase in mutations was also observed but at higher concentrations (between 0.25 and 3 mM or 47 and 562 μg/mℓ). However, as a result of the greater toxicity of EDB in the presence of S-9 the mutagenic activity was similar when expressed per 10^6 surviving cells (197 and 167 mutants/10^6 cell/mM for assays with and without S-9, respectively). In an earlier study, Clive (1973) reported that EDB induced mutations in mouse lymphoma cells diploid at the thymidine kinase (TK) locus. The effective dose for induction of $TK^{+/-}$ to $TK^{-/-}$ was 1-3 mM (188-562 μg/mℓ) in the absence of S-9. In a later study, Clive et al. (1979) reported that inclusion of S-9 increased the toxicity of EDB to the cells by ~10-fold. As was the case in CHO cells, on the basis of the lowest effective concentrations (LEC) for EDB, EDB was more mutagenic when assayed with S-9 (LEC = 45-50 μg/mℓ) than when assayed in the absence of S-9 (LEC = 150 μg/mℓ). Many undefined differences in these assay systems may account for these contrasting effects of S-9.

Mutation Assays in Higher Animals and Plants -- *Drosophila melanogaster* has been used in *in vivo* tests to assess the mutagenic potential of both ingested and gaseous EDB. Vogel and Chandler (1974) exposed Berlin K male flies to media containing 0.3 mM EDB for 2 days. Following exposure, males were mated with untreated females in order to produce three successive broods (two 3-day broods and one 4-day brood). The incidence of recessive lethals in the broods from treated males was 0.5, 1.49 and 1.30% as compared with 0.24, 0.21 and 0.36% for the respective controls. Since the increase in mutation frequency was most apparent in broods two and three, it was suggested that EDB affected spermatids and spermatocytes rather than mature sperm. This and other studies examining the mutagenicity of EDB in _D_. _melanogaster_ following exposure to the vapors are summarized in Table V-8.

Recently, Kale and Baum (1979, 1981, 1982) conducted a series of studies to assess the effects in _D_. _melanogaster_ of exposure to air pollutants. In the initial study (Kale and Baum, 1979), male flies were exposed to atmospheres containing 0.2-2 ppm of EDB for periods of between 11 and 100 hours. As many as 12 daily broods (grouped as broods 1-2, 3-6 and 7-12) were produced through the mating of exposed males. Increased incidences of sex-linked recessive lethal mutations were observed with this chronic exposure in all groups of broods. Although this indicated that mutations occurred in spermatozoa, spermatids and spermatocytes, the increase was least apparent in the spermatozoa. Similar results were reported by Kale and Baum (1981, 1982) when male flies were exposed to low levels of EDB for short periods of time (0.4 ppm for 2.6 hours, 0.5 ppm for 1.5 hours, and 0.7 ppm for 0.58 hours). When a high level (31.1 ppm) acute exposure (1.2 hours) was used however, the frequency of mutations in the spermatozoa was increased to a greater extent than in the other cell types. It was suggested that saturation of metabolic pathways could have accounted for the differences among effects observed after low level acute and chronic exposures and acute high level exposure.

TABLE V-8

Mutagenicity Assays of EDB in Higher Organisms

Species	Strain	Sex	Type of Assay	Route of Exposure	Concentration/ Dose	Duration of Exposure	Results	Comment	Reference
D. melanogaster	Berlin K	M	recessive lethal	oral (diet)	0.3 mM	2 days	+	Increased frequency of mutations were observed in second and third broods indicating that mature sperm were not affected.	Vogel and Chandler, 1974
D. melanogaster	Canton S	M	recessive lethal	inhalation	0.21 ppm	11 hours	+	These were considered chronic exposures. Mutations occurred in spermatozoa, spermatids and spermatocytes; however, spermatozoa cells were least affected.	Kale and Baum, 1979, 1981, 1982
					0.21 ppm	36 hours	+		
					0.25 ppm	72 hours	+		
					1 ppm	30 hours	+		
					1 ppm	60 hours	+		
					2 ppm	75 hours	+		
					2 ppm	100 hours	+		
D. melanogaster	Canton S	M	recessive lethal	inhalation	0.7 ppm	0.58 hours	+	These were considered acute exposures. The 31.1 ppm exposure resulted in a relatively greater increase in mutations in spermatozoa as compared with the other treatment groups.	Kale and Baum, 1981, 1982
					0.5 ppm	1.5 hours	+		
					0.4 ppm	2.6 hours	+		
					31.1 ppm	1.2 hours	+		
D. melanogaster	NR	M	recessive lethal	atmospheric exposure of eggs	1 ppm	3 hours	+	Mutations occurred in primordial spermatogonia.	Kale and Baum, 1983
					12 ppm	3 hours	+		
					50 ppm	1.5 hours	+		
					125 ppm	3 hours	+		
					125 ppm	3 hours	+		
					145 ppm	1.5 hours	+		
Mice	ICR/Ha Swiss	M	dominant lethal	i.p.	18 mg/kg	single injection	-	The dose was approximately the LD_5 and LD_{25} for each route.	Epstein et al., 1972
					90 mg/kg	single injection	-		
				gavage	50 mg/kg	5 days	-		
					100 mg/kg	5 days	-		
Mice	BDF_1	M	dominant lethal	gavage	100 mg/kg	5 days	-	Ethyl methanesulfonate was used as a positive control.	Teramoto et al., 1980
					150 mg/kg	5 days	-		
Rats	SD	M	dominant lethal	gavage	10 mg/kg	5 days	-	Same as above	Teramoto et al., 1980
					30 mg/kg	5 days	-		

NR - Not reported

In addition to studies using mature fruit flies, Kale and Baum (1983) exposed embryonic flies to EDB in order to assess damage to primordial spermatogonia. D. melanogaster eggs ~8-10 hours old were exposed to 1-145 ppm of EDB for 1.5-3.0 hours. After hatching, males were mated with untreated females and a dose-related increase in the incidence of sex-linked recessive mutation of between 0.2 and 4.2% was observed in the offspring of the treated males as compared with 0.05% in the controls. The incidence of mutations declined to 1.8% in the flies exposed to the highest level of 145 ppm for 1.5 hours. When 150 exposed males were bred successively for five broods, the clustering of mutations was supportive of damage to stem cells. It was concluded that embryonic spermatogonia were a sensitive target for EDB mutagenesis.

In mammalian systems, EDB has been tested in the dominant lethal assay with negative results reported in both rats and mice (Epstein et al., 1972; Teramoto et al., 1980) (see Table V-8). Epstein et al. (1972) exposed groups of 7-10 male ICR/HA Swiss mice to EDB by either a single i.p. injection of 18 or 90 mg/kg, or by 5 consecutive days of gavage treatment at doses of 50 or 100 mg/kg. The animals were subsequently mated with three untreated females/week for 8 weeks. Examination of the females during mid-pregnancy revealed no increases in dead implants as compared with the control animals. Using a similar experimental design, Teramoto et al. (1980) exposed groups of 15 male SD rats by gavage to EDB at doses of 10 or 30 mg/kg for 5 days followed by 10 weekly mating periods with 1 untreated female. In addition, groups of 7-9 male BDF_1 male mice were given five daily doses by gavage of EDB at 100 or 150 mg/kg followed by 6 weekly mating periods with 2 females. The mouse study was repeated twice. There were no increases in the incidence of dead implants in either mice or rats. In both the study by Epstein et al. (1972) and Teramoto et al. (1980) positive dominant lethal effects were observed with other compounds tested.

The plant Tradescantia has been used extensively as an indicator organism for the detection of volatile mutagenic compounds. Sparrow et al. (1974) and Nauman et al. (1976) used EDB to compare the induction of blue to pink mutations in three strains of Tradescantia. In the initial study (Sparrow et al., 1974), two clones, 02 and 4430, were tested, while in the latter report (Nauman et al., 1976) the data from clone 0106 were added. Exposure to the vapors of EDB at concentrations between 3.6 and 222.9 ppm for 6 hours resulted in a linear increase in pink events with increasing doses in clone 4430. Although the other two clones also produced linear dose-response relationships, these clones were less sensitive to EDB-induced mutations. There also were differences observed between the different clones with the other mutagens studied, ethyl methanesulfonate and ionizing radiation; however, the clone differences were not parallel between the three agents tested. Van't Hof and Schairer (1982) and Schairer et al. (1983) demonstrated that EDB mutations in Tradescantia were increased in a linear manner with length of exposure as well as concentration. It was demonstrated that EDB concentrations as low as 0.14 ppm resulted in detectable mutations following an extended exposure period (144 hours).

In an attempt to understand the mechanism that results in the great sensitivity of Tradescantia to the mutagenic action of EDB, Scott et al. (1978) investigated the activation of EDB by a metabolic activation system derived from plants. The indicator organism was the bacterium E. coli WP2 that undergoes reverse mutations to tryptophan-independent colonies.

Inclusion of an S-9 fraction derived from Tradescantia clone 4430 in the mutagenicity assay increased the number of revertant colonies from 13.4-372 revertants/10^6 surviving cells. For activation to occur, NADPH was needed in the incubation mixture indicating that an energy-dependent system was involved. An important inadvertent finding was that EDB mutagenicity increased slightly with the age of the solvent. Additional experiments demonstrated that freshly distilled EDB produced less revertants, both with and without metabolic activation, than the solvent as obtained from the supplier, and that the residue after distillation was highly mutagenic. The authors suggested that a high molecular weight contaminant was responsible for some of the mutagenic activity of EDB; however, further characterization of the contaminant was not performed.

Induction of Chromosomal Damage by 1,2-Dibromoethane -- Only limited information was available on the induction of chromosomal damage by EDB in cultured cells. Tezuka et al. (1980) exposed cultured Chinese hamster V79 cells to EDB for 26.5 hours at concentrations of 0, 0.5, 1, 2, 5 and 10 mM. The cells were examined for SCE, chromosomal aberrations and polyploidy at all but the highest concentration, which proved to be lethal. Both SCEs and chromosomal aberrations increased in a linear manner with dose. The aberrations were predominantly of the chromatid type and were similar to those observed in control cultures except for the higher frequency. The frequency of polyploid cells, triploids and tetraploids was elevated only at the highest nonlethal dose.

The only other data available are from two abstracts. Geddes et al. (1982) exposed human lymphocytes to 0.001-1000 μM (0.188-188 μg/mℓ) of

EDB for either 72 hours in the absence of S-9 or for 2 hours in the presence or absence of S-9. SCEs were induced under all conditions; however, the S-9 mix increased the response. Although no quantitative data were presented, it was indicated that the higher concentrations were cytotoxic. In the second abstract, Kristoffersson (1974) reported that EDB produced no chromosome breaks or changes in satellite association. No details were provided to evaluate this report.

Interactions with DNA -- A variety of different experimental systems have been used to evaluate whether EDB has a potential for interacting with nucleic acids. These systems include the preferential killing of bacteria deficient in DNA repair mechanisms, the inactivation of phage RNA, the measurement of DNA repair and the measurement and identification of DNA adducts. The results of these studies are presented in Table V-9.

Rosenkranz (1977), also reported in Brem et al. (1974), exposed *E. coli* strain pol A^+ and pol A^- (DNA repair proficient and deficient, respectively) to eight haloalkanes. The compounds were spotted on bacterial plates in amounts of 10 μℓ. All the haloalkanes tested (including EDB) preferentially killed the repair deficient cells as indicated by a greater zone of inhibition in this derivative. Results reported by Shiau et al. (1980), however, indicated no potential for DNA damage when EDB was tested in *B. subtilis*. In this study, 15 strains of *B. subtilis*, either recombination (Rec^-) or excision (Exc^-) repair deficient, were tested in a similar manner to that described above for *E. coli*, except that 50 μℓ were applied to the spot. Both in the presence and absence of a metabolic activation system, there were equal zones of growth inhibition between the

TABLE V-9

Interactions of EDB with DNA

Endpoint Studied	Indicator Organism	Method of Application	Dose	Results	Comment	Reference
Preferential inhibition of DNA repair deficient cells	*E. coli*	spot test	10 µℓ	+	The ratio of zones of inhibition for poly A^-/poly A^+ strains was 1.78.	Rosenkranz, 1977; Brem et al., 1974
Preferential inhibition of DNA repair deficient cells	*B. subtilis*	spot test	50 µℓ	-	15 different repair deficient strains were tested with negative results in strains.	Shiau et al., 1980
Unscheduled DNA synthesis	opossum lymphocytes	tissue culture	0.2-1879 µg/mℓ for 1 hour	+	The greatest increase was observed at 188 µg/mℓ, a metabolic activation system was not used.	Meneghini, 1974
Unscheduled DNA synthesis	human lymphocytes	tissue culture	2.5-10 µℓ/mℓ	+	The results were negative in the absence of S-9 and positive with activation, no dose-response relationship was apparent.	Perocco and Prodi, 1981
Unscheduled DNA synthesis	primary rat hepatocytes	tissue culture	0.001-0.01%	+	Positive responses were observed at 0.001 and 0.005%, although a dose-response relationship was not apparent.	Williams et al., 1982
Single strand DNA break	primary rat hepatocytes	tissue culture	6-564 µg/mℓ	+	The response was inversely related to the concentrations tested.	Sina et al., 1983
Single strand DNA break	rat liver	gavage	5-22 mg/100 g bw	+	Single strand breaks were time-dependent with maximal damage at 4 hours and complete repair at 96 hours after treatment with 75 mg/100 g bw.	Nachtomi and Sarma, 1977
Covalent binding to DNA	rat	gavage	2.6 µg/kg bw	+	Nonextractable radiolabeled EDB co-isolated with DNA.	Hill et al., 1978
Covalent binding to DNA	isolated DNA	*in vitro*	250 nmole	+	Binding occurred in the presence of S-9.	Banerjee and Van Duuren, 1979
Covalent binding to DNA	isolated DNA	*in vitro*	0.5 mM	+	The major adduct was S-[2-(N^7-guanyl)ethyl] GSH.	Ozawa and Guengerich, 1983

repair deficient strains and their isogenic wild type. The differences observed between the studies of Rosenkranz (1977) and Shiau et al. (1980) may be the result of the use of different bacterial species.

An indirect indication that a chemical has interacted with DNA is the initiation of unscheduled DNA synthesis (repair synthesis). In an early study, Meneghini (1974) exposed lymphocytes obtained from opossums to EDB for 1 hour at concentrations between 10^{-2} to 10^{-6} M (0.2-1879 μg/mℓ), and observed a subsequent increase in incorporation of ^{3}H-thymidine into DNA. Replicative synthesis was inhibited by hydroxyurea. The greatest increase in DNA synthesis was detected at a EDB concentration of 10^{-3} M. Perocco and Prodi (1981) performed a similar study using lymphocytes isolated from humans except that the incubation was conducted both with and without the inclusion of an S-9 metabolic activation system. The incorporation of ^{3}H-thymidine into DNA in the presence of hydroxyurea was slightly less than control values for cells treated with EDB at levels of 10, 5 or 2.5 μℓ/mℓ for 4 hours in the absence of S-9. In the presence of S-9, however, there was an increase in thymidine incorporation of ~47% at all doses with no dose-response relationship apparent. The low doses of EDB used in this study may have accounted for the weak response.

Williams et al. (1982) compared the induction of DNA repair synthesis in primary rat hepatocyte cultures by 30 chemicals, including EDB, with the reported carcinogenicity of these compounds. Primary hepatocytes undergo replicative DNA synthesis only rarely, and these cells still maintain a high degree of metabolic capability. After isolation, the cells were incubated for 18 hours with ^{3}H-thymidine and EDB at levels of 0.01, 0.005 or 0.001%

(not reported whether v/v or w/v). The high dose was toxic, but the two lower doses increased the number of grains/nucleus as observed by autoradiography. Of the 30 chemicals tested, a total of 12 were positive and 10/12 had been reported to be carcinogenic in animals.

In an abstract, Working et al. (1983) reported a dose dependent increase in unscheduled DNA synthesis in Fischer-344 rat hepatocytes and pachytene spermatocytes. The cells were exposed to 1-100 μM concentrations of EDB in culture for 18 hours (spermatocytes) or 4 hours (hepatocytes). In an in vivo study, rats were exposed to EDB by gavage at 10, 50 or 100 mg/kg. Two hours after treatment, hepatocytes and spermatocytes were isolated and assayed for unscheduled DNA synthesis. Unlike the in vitro study, there was no increase in the rate of unscheduled DNA synthesis after in vivo exposure.

Another indirect measure of chemical interaction with DNA is the demonstration of single-strand breaks in DNA produced as a result of excision repair or alkaline labile reaction products. Single strand breaks in DNA were assessed by Sina et al. (1983) by increased elution of isolated DNA through 2 μm membrane filters in alkaline solutions to assure strand separation. Freshly isolated rat hepatocytes were treated in culture with 0, 0.03, 0.3 or 3 mM (0, 6, 56 or 564 μg/mℓ) of EDB for 3 hours. DNA damage occurred at all dose levels; however, the lowest concentration was the most effective. These results could not be explained by decreases in cell viability. In this study, 91 chemicals were tested and there was a 92% correlation of chemicals causing single-strand breaks with known carcinogens and an 85% correlation of chemicals not causing single-strand breaks with known noncarcinogens.

Single strand breaks were assessed by Nachtomi and Sarma (1977) by analysis of alkaline sucrose gradient sedimentation profiles of DNA. Male Wistar rats were given a single gavage dose of EDB at levels of 0, 5, 7.5, 10, 15 and 22 per 100 g of body weight. The animals were killed 4 hours after treatment and the DNA from a portion of the liver was analyzed by alkaline sucrose gradient sedimentation. In the control animals, 84% of the DNA had molecular weights of $>3.2 \times 10^9$, while in the treated animals the percentage of high molecular weight DNA was 73, 39, 39, 35 and 35%, respectively. Using a single dose of EDB of 220 mg/kg bw, a progressive time-dependent increase in the percentage of lower molecular weight DNA was observed with treatment times between 0.5 and 24 hours. At a lower dose of 75 mg/kg bw, recovery of high molecular weight DNA indicated that DNA repair was complete by 96 hours post-treatment.

Hill et al. (1978) demonstrated directly that in vivo exposure to EDB results in covalent binding to DNA. Administration of 4.2 μmol of [u-^{14}C]-EDB to 300 g rats (2.6 μg/kg bw) was followed 24 hours later by the characterization of labeled macromolecules. Nonextractable radioactivity was associated with the DNA from all tissues examined (kidney, liver, spleen, small intestine, large intestine, stomach, testis, heart, brain and muscle in decreasing order of activity). In addition, Banerjee and Van Duuren (1979) demonstrated that in vitro incubation of radiolabeled EDB with isolated DNA and microsomes resulted also in the covalent binding of EDB to DNA. That this binding was microsomal enzyme-dependent was demonstrated through the use of varying amounts of microsomal protein, denatured microsomes or SKF-525A, a microsomal inhibitor. Addition of GSH to this system resulted in a 92-98% inhibition of radiolabel binding to DNA. On the other hand, Ozawa and Guengerich (1983), in DNA incubation experiments conducted without microsomes, demonstrated that addition of GSH greatly increased radiolabel binding to DNA if GSH transferase was also present. In this system, DNA became labeled whether the label was added at [u-^{14}C]-EDB or [^{35}S]-GSH, and the major DNA adduct was tentatively identified as S-[2-(N^{7}-guanyl)ethyl] GSH. These data clearly indicate that EDB can chemically react with DNA under appropriate conditions of metabolic activation.

Teratogenicity. Short et al. (1976) exposed groups of 18 pregnant Charles River CD rats and 13 pregnant CD-1 mice to 32 ppm EDB by inhalation for 23 hours/day on days 6-15 of gestation. Groups of 18 and 17 pregnant rats and 17 and 7 pregnant mice served as unexposed and feed restricted controls, respectively (Table V-10). The rats and mice were sacrificed on gestational days 20 and 18, respectively, and the fetuses were weighed and examined for external anomalies. One-half of the fetuses from each litter were examined microscopically for soft-tissue anomalies, and the remaining fetuses were examined for skeletal anomalies.

EDB exposure did not produce significant mortality in either the rats or mice (Short et al., 1976). The rats and mice gained significantly ($p<0.05$) less weight than the respective unexposed controls during the treatment period, but feed consumption also was significantly lower. In rats, EDB exposure was associated with significantly ($p<0.05$) reduced litter size (implants/dam and fetuses/dam) and significantly increased incidences of fetal hydrocephaly of the fourth ventricle and abnormal rib development (wavy ribs, lower frequency of 14th pair of ribs), but fetal weights were

TABLE V-10

Teratogenic Effects of EDB

Route (vehicle)	Species/ Strain	Exposure	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Inhalation (vapor)	rat/CD	0 (unexposed), 0 (unexposed feed-restricted) or 32 ppm[a], 23 hours/day	6-15	20	No significant mortality in 18, 17 or 18 control, feed-restricted or treated pregnant rats, respectively. Significantly decreased feed consumption and body weight in the treated group.	Implants/dam and fetuses/dam significantly ($p<0.05$) decreased in treated group. No effect on resorptions, fetal weight or sex ratio. Incidence of hydrocephaly (third ventricle) and wavy ribs increased ($p<0.05$), and 14th pair of ribs decreased ($p<0.05$) in treated group; soft-tissue or skeletal anomalies not found in feed-restricted group.[b]	Short et al., 1976
Inhalation (vapor)	rat/CD	0 (unexposed), 0 (unexposed feed-restricted), 20, 38 or 80 ppm[c], 23 hours/day	6-15	20	17, 15, 11, 15 and 16 pregnant rats in the control to high dose groups, respectively. Reduced ($p<0.05$) feed consumption at all exposure levels, weight loss ($p<0.05$) at 38 and 80 ppm, and 50% mortality at 80 ppm (0% in control).	Implants/dam and viable fetuses (0%, 96% in controls) reduced ($p<0.05$), dead fetuses and early resorptions insignificantly increased, and dams with complete resorptions increased ($p<0.05$) at 80 ppm. Reduced ($p<0.05$) fetal weight at 38 ppm (not determined at 80 ppm due to 0% live litters)[d]. No effect on sex ratio. Hematomas occurred in all treated fetuses, but data not given. Reduced ($p<0.05$) frequency of normally ossified centra at 20 ppm, but frequency increased ($p>0.05$) in the diet-restricted group.	Short et al., 1978

TABLE V-10 (cont.)

Route (vehicle)	Species/ Strain	Exposure	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Inhalation (vapor)	mouse/ CD-1	0 (unexposed), 0 (unexposed feed-restricted) or 32 ppm[e], 23 hours/ day	6-15	18	No significant mortality in 17, 13 or 9 control, feed-restricted or treated pregnant mice, respectively. Significantly decreased feed consumption and body weight in the treated group.	No treatment-related effect on litter size (implants/dam, resorptions, fetuses/dam), fetal weight or sex ratio. Significantly ($p<0.05$) increased incidence of skeletal anomalies (incompletely ossified supraoccipital and sternebrae, unossified incus and sternebrae) in treated group.[f] Same skeletal anomalies were increased in the diet-restricted group, but only incompletely ossified/ unossified sternebrae were significant ($p<0.05$).	Short et al., 1976
Inhalation (vapor)	mouse/ CD-1	0 (unexposed), 0 (unexposed feed-restricted) 20, 38 or 80 ppm[g], 23 hours/day	6-15	18	18, 9, 19, 17 and 19 pregnant mice in the control to high dose groups, respectively. 41 and 100% mortality at 38 and 80 ppm, respectively. Reduced ($p<0.05$) food consumption and body weight at 20 and 38 ppm.	Reduced ($p<0.05$) % viable fetuses and fetal weight, and increased ($p<0.05$) number of dams with complete resorption at 38 ppm. Increased ($p<0.05$) % late resorptions and decreased fetal weight at 20 ppm. No effect on sex ratio. Increased ($p<0.05$) incidence of runts, incompletely ossified supraoccipital, unossified incus, incompletely and unossified hyoid bone, and unossified phalanges at 38 ppm. 100% of sternebrae abnormally ossified (unossified, incomplete, split or extra ossification) at 38 ppm. Incompletely ossified supraoccipital and unossified incus at 20 ppm. Diet-restricted control data not available[h].	Short et al., 1978

TABLE V-10 (cont.)

Route (vehicle)	Species/ Strain	Exposure	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Intraperitoneal (corn oil)	rats/ Sprague-Dawley	0 (vehicle control) or 55 mg/kg/day, single injection	1-15	21	Unspecified changes in the weights of two unspecified organs. No effect on body weight.	Results not reported in detail (preliminary study). No effect on pre- or post-implantation survival, fetal body weight or length. No external or gross or visceral anomalies.	Hardin et al., 1981

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[a] Mean feed consumption in the untreated, feed-restricted and treated groups were 21.1, 5.0 and 10.5 g/rat/day, respectively.

[b] The number of fetuses examined for external, soft-tissue and skeletal anomalies in the unexposed, feed-restricted and treated groups were 269, 207 and 259; 129, 99 and 116; and 140, 108 and 135, respectively.

[c] Mean feed consumption in the untreated, feed-restricted and treated groups was 19, 4, 15, 9 and 3 g/rat/day, respectively.

[d] The number of fetuses examined for external, soft-tissue and skeletal anomalies in the untreated, feed-restricted and treated groups was 227, 112 and 115; 185, 88 and 97; 146, 71 and 75; 184, 88 and 96; and 185, 88 and 97, respectively.

[e] Mean feed consumption in the untreated, feed-restricted and treated groups was 5.6, 3.6 and 3.5 g/mouse/day, respectively.

[f] The number of mouse fetuses examined for external, soft-tissue and skeletal anomalies in the unexposed, feed-restricted and treated groups were 155, 75 and 80, 81, 41; and 36, 17 and 19, respectively.

[g] Mean feed consumption in the untreated, feed-restricted and treated groups was 6.3, 0.9, 3.9, 2.3 and 0.8 g/mouse/day, respectively.

[h] The number of fetuses examined for external, soft-tissue and skeletal anomalies in the untreated and treated groups was 208, 98 and 110; 218, 103 and 115 and 44, 20 and 24; examination of feed-restricted fetuses precluded due to high maternal lethality (cannibalism) and embryo/feto lethality.

not affected significantly by exposure. Increased incidences of skeletal anomalies were not observed in fetuses from the rats with a restricted feed intake. Fetal weights were significantly ($p<0.05$) lower in the EDB-exposed mice. Increased incidences of external or soft-tissue anomalies were not found in the fetuses from the EDB-exposed mice, but abnormal ossification was prevalent (significantly increased incidences of incompletely ossified supraoccipital, unossified incus and incompletely/unossified sternebrae). The frequency of these skeletal anomalies also was elevated in the fetuses from the mice that were on the restricted feed intake, suggesting that these skeletal effects may be attributable to malnourishment rather than to direct EDB toxicity.

In a similarly designed follow-up study, groups of 11-17 pregnant Charles River CD rats and 9-19 CD-1 mice were exposed to 0 (unexposed control), 0 (unexposed feed-restricted controls), 20, 38 or 80 ppm EDB, 23 hours/day on days 6-15 of gestation (Short et al., 1977, 1978) (see Table V-10). Maternal sacrifices and fetal examinations were conducted as described previously (Short et al., 1976).

In rats, EDB-related maternal toxicity was indicated by significantly ($p<0.05$) reduced feed consumption at all exposure levels, by significant weight loss at 38 and 80 ppm, and mortality (50%) at 80 ppm. A significant reduction in the number of implants and viable fetuses (0%), an increased percentage of dead fetuses and early resorptions, and an increased number of dams with complete resorptions also occurred at 80 ppm, indicating pronounced embryotoxicity. The only evidence of embryotoxicity at lower exposures was a significant reduction in fetal weight in the 38 ppm group.

Hematomas were the most common external malformation in the fetuses of the treated rats and reportedly occurred in all groups (data not provided), but soft-tissue anomalies were not present in any of the treated rats at statistically significant levels. Skeletal examination revealed a significantly reduced percentage of fetuses with normally ossified centra in the 20 ppm group, but the frequency of this effect was only insignificantly reduced at 38 ppm.

In mice, high maternal mortality occurred in the 38 and 80 ppm groups (41 and 100%, respectively), and feed consumption and maternal body weight were significantly decreased at 20 and 38 ppm. Fetotoxic effects were indicated at 20 ppm by a significantly increased percentage of late resorptions and reduced fetal weight, and at 38 ppm by a decreased percentage of viable fetuses, a high frequency of dams with complete resorptions (60%), and decreased fetal weight. There was a significantly elevated incidence of runts (which was categorized as a soft-tissue anomaly) among the fetuses in the 38 ppm group, but it was noted that this may be a reflection of the reduced body weight/maternal feed consumption/weight gain of the dams. Treatment-related external and other soft-tissue anomalies were not reported in the mice, but a statistically significant increase in the incidence of fetal skeletal anomalies was found at 20 ppm (incompletely ossified supraoccipital, unossified incus) and 38 ppm (incompletely ossified supraoccipital, unossified incus and abnormal ossification in the hyoid bone, sternebrae and phalanges as detailed in Table V-10). There were insufficient numbers of fetal mice to evaluate the effect of feed restriction on skeletal development (due to maternal lethality and a significantly low percentage of viable fetuses and high frequency of resorptions), but the results of the previous study (Short et al., 1976) suggest that some of the effect (i.e., sternebrae anomalies) may be related to nutrition.

Daily i.p. injection of EDB (corn oil vehicle) to Sprague-Dawley rats at a dose of 55 mg/kg on days 1-15 of gestation produced signs of maternal toxicity (change in two unspecified organ weights), but no evidence of fetotoxicity or teratogenicity (Hardin et al., 1981). The results of this study are not reported in detail because this experiment was part of a preliminary survey with other occupational chemicals. It was noted that each group (treated and vehicle control) consisted of 10-15 inseminated females, that the dose represented the maximum tolerated maternal dose as established in a preliminary study, and that the animals were sacrificed on day 21 of gestation. The criteria for maternal toxicity were significantly reduced body weight gain or altered weights (absolute or relative) of two or more organs, gross pathologic changes of the maternal organs, or histologic changes in the brain, heart, lungs, liver, spleen, kidneys, adrenals or ovaries. Fetotoxicity was established by reduced pre- or postimplantation survival or reduced fetal body weight or length. Teratogenicity was evaluated by external examination (all fetuses), gross visceral examination (one-half to two-thirds of the fetuses) and gross skeletal examination (remaining fetuses).

Reproductive Effects. Studies with several mammalian species indicate that EDB may produce sperm abnormalities. The bull appears to be particularly susceptible to reversible effects on spermatozoa when EDB is administered by repeated oral doses or peritesticular injection. Rams, chickens and rats are apparently more resistant to EDB-induced sperm damage. Species differences in susceptibility to sperm damage from EDB exposure have been the focus of a series of investigations that began in the 1960s and continues to the present time.

Amir and Volcani (1965) first reported the results of feeding EDB to Friesian bulls at an average daily dose of 2 mg/kg beginning from the age of 4 days (4 mg/kg every other day). When the four treated bull calves reached the age of 14-16 months, weekly semen collections revealed a low sperm density with poorly motile spermatozoa displaying various structural abnormalities. After a 10-day (one animal) or a 3.5-month (one animal) period free from exposure to EDB, sperm production returned to normal. A similar pattern of reversible damage was seen in bulls exposed to EDB beginning at age 14-16 months. Antispermatogenic effects were evident as early as 2 weeks after initiation of treatment at a dose of 2 mg/kg/day, persisting for a month after treatment was discontinued. Complete recovery took 2-3 months. Histopathological examination of the testes and epididymis from hemi-castrated bulls treated with EDB from calfhood to age 17-22.5 months showed a depopulation in the majority of the seminiferous tubules (Amir and Volcani, 1967). Evidence of recovery of tubule function in the remaining testis was obtained in 2 of the 3 bulls when the animals were slaughtered 7 months later. Repeated semen sampling at regular intervals between cessation of treatment and slaughter revealed complete recovery in two bulls within 3-4 months, with sperm density and mobility remaining below normal in the third bull for several months. Sexual libido was not affected by EDB treatment.

In subsequent studies, EDB administered orally to mature Friesian bulls in 10 doses of 4 mg/kg given on alternate days, or given as a single 110-120 mg injection into the peritoneal fluid surrounding the testis, induced a high percentage of abnormal spermatozoa in ejaculates collected 12-17 days after start of treatment (Amir and Ben-David, 1973). These malformations persisted for ~1-2 months after dosing was discontinued. Radioactivity derived from [^{3}H]- or [^{14}C]EDB could be found in spermatozoa collected from ~1 week after the first oral dose (7 or 10 doses of EDB at 4 mg/kg on alternate days) until 2-3 weeks after treatment ceased (Amir, 1973). The results indicated that the spermicidal action of EDB is not directly on the sperm cells, but occurs during the process of spermiogenesis and sperm maturation (nota bene, the mean duration of epididymal transit of the spermatozoa in the bull is 11 days). This conclusion was supported by evidence showing that the percentage of sperm abnormalities reached its highest level when little EDB-derived radioactivity could be detected in the sperm. In addition, the effect of EDB on sperm concentration was much more pronounced in adult bulls (4.5-5 years old) than in young bulls (15-24 months old), and the period required for recovery was longer in adult animals (Amir, 1975).

An attempt was made to understand the effects of EDB on sperm viability in the Friesian bull by examining protein changes in epididymal and ejaculated spermatozoa (Amir and Lavon, 1976). Seven young bulls (15-18 months old) and three old bulls (4.5-5.5 years old) were treated orally with 10 doses, given on alternate days, of EDB at 4 mg/kg/dose. No significant changes were found in the total nitrogen, amino acid or lipoprotein contents of the spermatozoa collected either from the epididymis 1 day after the last dose (young bulls), or from ejaculates collected 9-13 days after the end of the treatment (old bulls). Ejaculates collected from the old bulls showed a 100% incidence of spermatozoa with misshapen heads, and a high percentage of degenerative changes. In only one of the four young bulls were there spermatozoa with abnormally shaped heads in the caput and cauda epididymis. The results suggested that spermatozoa affected by EDB may undergo further damage during transit through the genital tract. Changes were observed in the amino acid composition of the proteins of spermatozoa from both the epididymis and the ejaculates. Amir and Lavon (1976) suggested that EDB may bind to thiol groups in nuclear basic proteins of bull spermatozoa that are known to be rich in cysteine residues. Adduct formation of this type could be expected to interfere with the normal binding of the protein molecule to the DNA, causing improper packing of the chromatin. A follow-up study established that the misshapen spermatozoa collected from EDB-treated bulls contained reduced amounts of DNA and protein (Amir et al., 1977).

Amir et al. (1979) subsequently examined the time-course of development for sperm damage in order to establish more clearly the exact stage at which germ cells are affected by EDB. Seven Friesian bulls (16-18 months old) and two Friesian bulls (13 months old) were given a single 270 mg peritesticular injection of EDB and castrated 3, 4 or 5 days later. Measurements of protein and DNA content of the spermatids indicated that EDB affects the spermatids during the phase of nuclear elongation.

Initial attempts to produce antispermatogenic effects in sheep by oral exposure to EDB were unsuccessful (Amir and Ben-David, 1973). After 4 months of treatment of several rams of the Awassi and German mutton Merino breeds, toxic rather than antispermatogenic effects were produced by the administration of increasing doses of EDB (dose not reported). On the other hand, ElJack and Hrudka (1979) succeeded in producing transient sperm abnormalities by repeated s.c. injections of relatively high doses of EDB. Colombian rams (18-30 months old) received 12 consecutive daily injections of EDB at a dose of 7.8, 9.6 or 13.5 mg/kg/day. Acute systemic toxicity was evident at the 16.9 mg/kg/day dose level and thus was not used for this study. Sperm production was normal in all treated groups for up to 2-3 weeks following EDB administration. A dose-related decline in sperm motility and dose-related abnormalities of the acrosome (the predominant effect seen in this study) began to appear during the fifth week after initiation of treatment. Nuclear abnormalities and mitochondrial sheath defects were also seen under phase-contrast or electron microscopy. Sperm damage was maximal between 9 and 12 weeks and disappeared 15 weeks after beginning treatment. The absence of adverse effects during the first 4 weeks after beginning treatment indicated that EDB was not spermidical and did not act directly on the differentiating sperm organelles. Considering the temporal relationship between the period of EDB treatment and the time of sperm transit through the epididymis, the length of spermiogenesis, meiosis, and spermatogonial differentiation, the authors concluded that the effect of EDB was on the diploid precursors of the sperm, presumably during their DNA synthesis.

More recent studies by Amir et al. (1983) are not entirely supportive of the results of ElJack and Hrudka (1979). A single peritesticular injection of EDB was administered to rams to allow for a more precise determination of the time, site and stage at which cells are affected by examination of the injected testis and epididymis removed at different times. Nine crossbred

Awassi X Finnish Landrace rams (seven rams 8-10 months old, two mature rams) and 14 Corriedale rams (16-30 months old) received a single injection of either 430 or 250 mg EDB, and the effect of treatment on germ cells, epididymis and epididymal spermatozoa followed as a function of time after injection up to 120 hours. Sperm motility and sperm density in the ejaculate also was evaluated. In the testes, a high incidence of misshapen and vacuolized decondensed elongating and elongated spermatids was observed at 48 hours after treatment, with damage being evident as early as 24 hours. Effects at the 250 mg dose were less severe than at the 430 mg dose of EDB. No adverse effects on the premeiotic stages of spermatogenesis were indicated (evaluation criteria not stated). In the epididymis, the epithelium of the corpus and cauda segments was damaged in the majority of treated rams at both doses 48 hours after injection. The spermatozoa in these segments were abnormal, mainly with acrosomal defects, when evaluated 48-120 hours after injection. In the ejaculates from rams given 250 mg of EDB, sperm density was low and large percentages of the spermatozoa showed acrosomal and tail abnormalities. These effects became evident as early as 2 or 3 days after injection in two of the three treated rams, but did not reach maximum severity in the third treated ram until 12 days after injection. In all cases, the effect of EDB treatment was reversed several weeks after injection.

The effect of EDB exposure on spermatogenesis in the rat has not been studied directly. However, several investigations indicate that reproductive performance in the rat can be impaired by EDB at high doses. Short et al. (1979) exposed groups of 10 Charles River CD rats to 0, 19, 39 or 89 ppm of EDB in air, 7 hours/day, 5 days/week for 10 weeks. Adverse effects were seen early in the group exposed to 89 ppm EDB. These animals developed atrophy of the testis, epididymis, prostate and seminal vesicles. None of the rats from this high dose group were able to impregnate female rats successfully during a 2-week mating period beginning immediately after termination of exposure. Because mortality and other signs of morbidity also occurred among rats exposed to 89 ppm EDB, it is not possible to conclude that the reproductive system of rats is uniquely susceptible to damage. Nevertheless, EDB-induced testicular atrophy also has been observed in chronic bioassays (see Carcinogenicity Section) with Osborne-Mendel rats exposed by gavage (27.4 and 29.1 mg/kg/day, TWA) (NCI, 1978), in Sprague-Dawley rats exposed by inhalation (20 ppm, 7 hours/day, 5 days/week for 18 months) in combination with disulfiram in the diet (Wong et al., 1982), in Fischer 344 rats exposed by inhalation (10 and 40 ppm, 6 hours/day, 5 days/week for 88-103 weeks) (NTP, 1982) and in $B6C3F_1$ mice exposed by gavage (76.6 mg/kg/day for 53 weeks) (NCI, 1978).

In addition, EDB has been demonstrated to adversely affect egg laying in hens. Bierer and Vickers (1959) fed rats fumigated the day before with a commercial fumigant containing EDB (EDB levels not reported) to laying hens for 10 days. Egg number and size started to decline before cessation of treatment and continued to decline over the next 2 weeks of nonexposure. Egg laying was not returned to normal after 6 weeks. Similar results were observed by Caylor and Laurent (1960) with White Leghorn hens fed oats fumigated with 64% carbon tetrachloride, 26% ethylene dichloride and 7% EDB. Of the three components of this fumigant formulation, Fuller and Morris (1962) demonstrated that EDB was the active ingredient. White Leghorn hens were administered by gavage EDB at doses of 0, 0.5, 1, 2, 4, 8 or 20 mg/bird/day for up to 36 weeks. At 12 weeks, egg size was decreased at dose levels ≥ 1 mg/bird/day, and laying had stopped in the high dose group. Also in the hens of the high dose group, body weight and food consumption were decreased, and mortality was increased. The other components of the fumigant, carbon tetrachloride and ethylene dichloride, were inactive when tested individually.

Summary

Limited data are available regarding the acute toxicity of orally administered EDB, but single-dose LD_{50}s have been reported in the range of 117-146 mg/kg for rats, 420 mg/kg for mice, 55 mg/kg for rabbits, and 110 mg/kg for guinea pigs (Rowe et al., 1952). Single oral doses of 110-120 mg/kg have produced pathological (e.g., hepatomegaly, centrilobular necrosis) and biochemical (e.g., increased levels of liver lipids, increased levels of serum enzyme, evidence of DNA damage and repair) indications of hepatotoxicity in rats (Nachtomi et al., 1968; Nachtomi and Alumot, 1972; Broda et al., 1976; Nachtomi and Farber, 1978; Nachtomi and Sarma, 1977; Nachtomi, 1980). Administration of EDB at a dose of 10 mg/kg/day by gavage produced elevated SGPT and SDH levels after 12 days of administration (Nachtomi, 1980). Symptoms of toxicity and effects on tissues other than the liver were not described in any of the acute oral studies with rodents, but CNS depression has been reported to be a primary effect of lethal single oral exposures in dogs (Kistler and Luckhardt, 1929).

Maximum survival exposures in rats inhaling EDB vapor ranged from 10,000 ppm for 1.2 minutes to 100 ppm for 16 hours, and exposure to 50, 100 or 200 ppm EDB for 7, 2.5 and 0.7 hours, respectively, reportedly did not produce evidence of toxicity in the same species (Rowe et al., 1952). Adverse effects were not observed in rats or guinea pigs that were subjected to 13 seven-hour exposures of 25 ppm EDB in 16-17 days (Rowe et al., 1952). Deaths in multiple species of rodents that resulted from acute inhalation exposure to EDB vapor were usually attributed to respiratory and cardiac failure, and effects typically were described in the lungs (e.g., congestion, edema, hemorrhages), liver (e.g., centrilobular degeneration and necrosis) kidneys (e.g., congestion edema, cloudy swelling) and spleen (i.e., congestion); central nervous system effects (i.e., depression) were most pronounced at the higher concentrations (lethal exposures).

Acute dermal studies with rats and rabbits have shown that EDB can be absorbed through intact skin in amounts that can elicit local and systemic toxic effects, as well as death (Thomas and Yant, 1927; Rowe et al., 1952).

Administration of EDB to rats and mice at TWA doses of ~28-77 mg/kg/day by gavage for 49-61 weeks produced carcinomas of the forestomach (proximal tumors) as well as carcinomas of the lungs, liver and thyroid, and hemangiosarcomas of the circulatory system. Early mortality that may have been tumor-related (forestomach carcinoma) (NCI, 1978) was observed, but this diagnosis is not certain, however, because of the toxic effects of EDB at these doses. Non-neoplastic lesions and/or neoplasms were induced in the forestomach (hyperkeratosis, acanthosis and squamous cell carcinoma in rats and mice), liver (degenerative/inflammatory alterations and hepatocellular carcinoma in rats), adrenal cortex (degeneration in rats), circulatory system (hemangiosarcomas in rats) and testis (atrophy in rats and mice).

Subchronic inhalation exposure to EDB at levels of 25 ppm reportedly did not produce adverse effects on behavior, growth, body or organ weights, BUN or tissue histology in rats, guinea pigs, rabbits or a limited number of monkeys, although mortality that was caused by respiratory infection occurred in the rats and guinea pigs (Rowe et al., 1952); similar exposure to 50 ppm EDB produced effects such as decreased growth rate and organ weight/histological effects in the lungs, liver, kidneys and testes. Chronic inhalation exposure to 20-40 ppm EDB produced mortality in rats and mice, with non-neoplastic and neoplastic lesions in the respiratory system (inflammation and hyperplasia/metaplasia of the nasal cavity/trachea/lung, adenoma/carcinoma/adenocarcinoma of the nasal cavity or lung), liver (necrosis in rats), kidney (toxic nephropathy in rats), circulatory system (hemangiosarcomas, particularly in the spleen of rats and mice), mammary gland (adenomas and carcinomas in rats and mice), testis (atrophy in rats and mice), tunica vaginalis (mesothelioma in rats), pituitary (adenoma in rats), adrenals (cortical degeneration and tumors in rats) and subcutaneous tissue (fibrosarcoma in female mice) (NCI, 1978; NTP, 1982; Stinson et al., 1982; Wong et al., 1982). Three times/week administration of EDB for life to the skin of mice caused increased incidences of local skin papillomas as well as distant lung papillomas, but skin initiation with EDB followed by promotion with phorbol myristate acetate did not induce these tumors (Van Duuren et al., 1979).

A large number of studies have been conducted evaluating the mutagenic potential of EDB in a variety of *in vitro* and *in vivo* test systems (Table V-7). In bacterial systems EDB consistently produced both reverse and forward mutations. The addition of metabolic activation by the incorporation of an S-9 mix into the assay produced variable results. In some studies, metabolic activation enhanced the mutagenic response, while other studies showed no change or a decrease in mutagenic activity. Similar results were reported for *in vitro* assays using eukaryotic cells. Some of the variability in the response of EDB may be the result of its high volatility.

Only in a single study has there been an indication that a contaminant of EDB may account for some of the observed mutagenic activity. Scott et al. (1978) observed increasing mutagenic activity in *S. typhimurium* with increasing age of the EDB tested. Following distillation the activity in the distillate was decreased, while there remained high activity in the residue. Although this suggests that a high molecular weight contaminate of EDB was mutagenic, the freshly distilled EDB was also still mutagenic when metabolic activation (plant extract) was provided.

In vivo exposure of higher organisms to EDB also has resulted in mutations. Sparrow et al. (1974), Nauman et al. (1976), Van't Hof and Schairer (1982) and Schairer et al. (1983) have shown that the plant, *Tradescantia*, was a very sensitive indicator organism for the detection of gaseous mutagens, and that EDB produced mutations in this plant that were linearly related both to time and concentration. Both oral (Vogel and Chandler, 1974) and inhalation (Kale and Baum, 1979, 1981, 1982) exposure of adult male fruit flies, *D. melanogaster*, to EDB resulted in increases in recessive lethal mutations in the offspring. Also, in fruit flies, EDB has been demonstrated to cause mutations in primordial spermatogonia when exposure occurred to the embryo (Kale and Baum, 1983). In mammals, however, EDB has failed in the induction of dominant lethal mutations when tested in mice (Epstein et al., 1972; Teramoto et al., 1980) or rats (Teramoto et al., 1980).

Only limited data are available on the clastogenic effects of EDB. Tezuka et al. (1980) observed an increase in cultured V79 cells of SCE, chromosomal aberrations, and cell polyploidy. Geddes et al. (1982) reported in an abstract that EDB increased SCEs in cultured human lymphocytes, but only in the presence of S-9. In a second abstract, Kristofferson (1974) reported no clastogenic effect of EDB; however, insufficient data were provided to evaluate this study.

Interactions of EDB with DNA have been demonstrated by both indirect and direct methods. Brem et al. (1974) and Rosenkranz (1977) observed an increased sensitivity to the lethal effects of EDB in DNA repair deficient strains of *E. coli*, while Shiau et al. (1980) failed to obtain similar results with DNA repair deficient strains of *B. subtilis*. The differences in the results of these two assays can not be easily resolved. In other indirect assays of DNA damage, Meneghini (1974), Perocco and Prodi (1981) and Williams et al. (1982) observed increased unscheduled DNA synthesis in cultured mammalian cells following exposure to EDB, and Sina et al. (1983) and Nachtomi and Sarma (1977) observed EDB-induced single strand breaks in DNA *in vitro* in cultured cells and *in vivo* in rat liver, respectively. Direct evidence of interactions with DNA has been provided by the formation of nonextractable radiolabeled DNA following both *in vivo* and *in vitro* exposure to radiolabeled EDB. Ozawa and Guengerich (1983) tentatively identified the major EDB DNA adduct as S-[2-(N^7-guanyl)ethyl] GSH.

The antispermatogenic effect of EDB was recognized first in bulls nearly 20 years ago (Amir and Volcani, 1965). Reversible spermatogenic effects were produced in bulls by subacute and longer oral administration and by peritesticular injection of a single dose of EDB (Amir and Ben-David, 1973; Amir, 1975; Amir et al., 1977, 1979). It has been postulated that the action of EDB occurs during the process of spermiogenesis and of sperm maturation and is not a direct effect on the spermatozoa (Amir, 1973; Amir et al., 1979). The reversible antispermatogenic effect of EDB seen in bulls also has been produced experimentally in rams by repeated s.c. injection (ElJack and Hrudka, 1979) and by single peritesticular injection (Amir et al., 1983), but not by chronic oral administration. Rats may be relatively resistant to the spermicidal action of EDB, since adverse effects on fertility are produced only at levels of exposure associated with systemic toxicity (Short et al., 1979; Amir et al., 1983). Nonetheless, testicular atrophy has been demonstrated in both rats (NCI, 1978; Wong et al., 1982; NTP, 1982) and mice (NCI, 1978) chronically exposed to EDB.

The teratogenic potential of EDB has been evaluated only in rats and mice that were exposed to EDB by inhalation (Short et al., 1976, 1978), or rats exposed by i.p. injection (Hardin et al., 1981). The inhalation studies showed that nearly continuous inhalation exposure (23 hours/day) to 20 ppm EDB vapor on days 6-15 of gestation produced skeletal anomalies (e.g., reduced frequency of normally ossified centra, incompletely ossified supraoccipital, unossified incus) in both species with reduced maternal feed consumption. Similar exposure to high concentrations of EDB (32-80 ppm) produced more pronounced skeletal anomalies, limited evidence of soft-tissue anomalies (i.e., hydrocephaly in rats at 32 ppm), and dose-related maternal and fetotoxicity. Intraperitoneal injection of EDB on days 1-15 of gestation reportedly did not produce fetotoxicity or external, gross or visceral abnormalities in rats at a dose (55 mg/kg) that produced changes in maternal organ weights (but not in maternal body weight).

VI. HEALTH EFFECTS IN HUMANS

Clinical Case Studies

Case studies regarding incidents of human exposure to EDB are largely anecdotal in nature. The earliest reported study involved the death of a woman who was mistakenly administered the contents of a 70 g bottle of EDB through a gauze mask in an attempt to induce anesthesia (Marmetschke, 1910).

Lesions resulting from dermal contact with EDB have been recognized for many years, prompting a series of controlled experiments with human volunteers. Nine volunteers rubbed 1 mℓ of a fluid containing 55 ppm EDB into their hands for 1 minute, and 2 hours later washed the area with soap and water (Pflesser, 1938). No adverse symptoms developed, and the experiment was repeated using the same volunteers and again obtaining negative results. When the same 1 mℓ dose of the fluid was soaked into a cotton swab and held in contact with the skin for 2 hours using the same volunteers, all subjects became ill and developed blisters and swelling at the site of contact. Additional experiments using pure EDB confirmed that severe skin reactions developed from direct contact with the substance, the severity of which was increased if the application site was covered (which presumably reduced evaporation of the EDB). Pflesser (1938) indicated that EDB was a potential skin sensitizer as well as a primary skin irritant.

Only one case report of EDB poisoning has involved ingestion (Olmstead 1960). The authors described the case of a 43-year-old woman who had ingested 4.5 mℓ of EDB in order to commit suicide. The patient died 54 hours after ingesting the substance. At autopsy, the major findings were a massive central lobular necrosis of the liver and focal proximal tubular epithelial damage in the kidney.

Epidemiological Studies

The mortality experience of 156 male workers that were exposed to EDB in two EDB manufacturing facilities was investigated by Ott et al. (1980). The emphasis of this study was on cancer mortality and respiratory disease (emphysema/chronic bronchitis/asthma and influenza/pneumonia). Fifty-seven of the workers were reactor and still operators in a plant (Plant A) that operated continuously from the mid-1920's until 1976. Industrial hygiene surveys that were conducted in Plant A during 1950-1952 showed breathing zone concentrations that ranged from 1-31 ppm, with excursions as high as 71 ppm after spills. Extensive monitoring conducted during 1971-1972 and 1975 indicated TWA concentrations of 2-5 ppm, with occasional suspected excursions above 75 ppm (based on historical observations of strong odor detection and acute respiratory irritation at ~75 ppm). The employees of Plant A were, however, reportedly potentially exposed to 25 other substances including bromine, benzene, substituted phenols, vinyl bromide, carbon tetrachloride and many other halogenated hydrocarbons. Bromide was detected in the blood at a mean level of 6.5 mg/100 mℓ (range, 1-17 mg/100 mℓ) in selected Plant A reactor and still operators between 1957 and 1970 (2-5 men/operation/year every 2-4 years), but this finding is not specific for EDB in the presence of other process chemicals that contain bromine. Plant B was operated from 1942-1969 and also consisted of reactor and distillation operations, but monitoring data were not available. Exposures in Plant B were considered to be primarily to EDB, bromine, ethylene, sulfur dioxide and chlorine.

Workers with likely EDB exposure from both Plants A and B were identified by reviewing annual plant census lists and selecting all reactor and still operators from Plant A, and selecting all lead burners (who could have spent 90% of their time in the EDB production area), reactor and still operators and foremen from Plant B (Ott et al., 1980). Workers in Plant A who left employment before 1940 were not included in the study, which was due in part to the difficulty of conducting social security follow-up (follow-up of employees who left the company after this date was conducted primarily by the Social Security Administration). Five workers from Plant A that were exposed to arsenicals in addition to EDB were excluded from mortality analysis. Because of limited exposure data, duration was the only indicator of exposure used in the analysis; the numbers of workers from Plant A that were exposed for <1 year, 1-5 years and ≥6 years were 7, 39 and 16 and from Plant B were 24, 45 and 30, respectively for the same time periods. Expected deaths were calculated from observed mortality rates for United States white males, by 5-year groups, for the years 1942-1971.

Mortality analysis showed that deaths from malignancy and respiratory infection occurred more frequently than expected among the workers in Plant A; however the increases that were due to malignancy were not statistically significant ($p<0.05$) (Ott et al., 1980). Deaths from total malignant neoplasms occurred in 5 cases vs. 2.2 expected ($p<0.072$); deaths from malignant neoplasms of the digestive system occurred in 2 cases vs. 0.7 expected ($p<0.156$); deaths from all malignant neoplasms other than digestive system occurred in 3 cases vs. 0.9 expected ($p<0.063$); and deaths from influenza and pneumonia occurred in 2 cases vs. 0.3 expected ($p<0.037$). There were fewer deaths than expected from malignant neoplasms (2 vs. 3.6 expected) and other causes in Plant B, with the exception of cardiovascular system diseases (11 vs. 9.1 expected). No statistically significant increase in deaths was found when mortality was examined in relation to duration of exposure and interval since first exposure for both units (Plants A and B) combined, although a statistically insignificant ($p<0.05$) increase in mortality from cancer (total malignancies) among employees with more than 6 years' exposure was apparent. Although the above data do not suggest an increased risk of death from malignant neoplasms or respiratory disease, the results should be regarded as equivocal because interpretation is complicated by the small population size, poorly characterized exposure concentrations, and concomitant exposure to numerous other chemicals.

Unpublished mortality data have been submitted to the U.S. EPA concerning workers at two plants in England involved in EDB manufacture (Turner, 1976). At a plant in Amlwch, England, the vital status was determined at the end of 1975 for 273 out of a total of 297 employees. The cohort consisted of 212 persons who were employed as of 1959, 22 persons who joined the plant between 1959 and 1969, and the remainder not specified. The average experience of the 31 workers who had died by 1975 was 13 years each. No details regarding EDB exposure concentrations were given. In comparison with the death rate for unexposed males 45-64 years of age who had lived in the vicinity of the plant, no excess was found in deaths from cancer, heart disease, cardiovascular disease, or all causes combined. The authors acknowledged that the small cohort and lack of exposure data made it difficult to formulate any firm conclusions regarding EDB.

A second unpublished mortality survey involving a EDB manufacturing plant in Hayle, England, also was submitted to the U.S. EPA (Turner, 1977). The vital status was determined for 117 men who were potentially exposed to

EDB for at least 4 years during the period 1940-1970. The initial list of candidates for the study totaled 242 individuals. Although levels of exposure to EDB were not reported, subsequent estimates (Ter Haar, 1981) placed the average airborne concentration at ~10 ppm. The authors acknowledged the difficulties of calculating death rates from such small numbers, but concluded nevertheless that no excess of cancer deaths could be found in comparison with males in the same age ranges in Southwest England.

Evidence of an antispermatogenic effect from the administration of EDB to animals has prompted the search for similar effects in exposed human populations. Most of the results of this effort are available only in unpublished form. All of the available studies are limited in their design and scope. One industry-sponsored study has been published that provides the results of a retrospective cross-sectional study of the fertility experience of workers at four EDB manufacturing plants in the United States (Wong et al., 1979). Comparisons were made of the number of children born to the wives of 297 EDB-exposed workers vs. expected births based upon national female fertility rates for the period 1958-1977. Exposures to EDB at three of the plants were grouped into high (0.5-5.0 ppm) and low (<0.5 ppm) categories, and included a total of 1092 person-years at risk. Matched controls were not included in the study. At none of the three plants where exposure data were available was there a significant difference in the ratio of observed/expected births. At the fourth plant a total of 13 births was observed vs. 25.51 expected, which was significant at the p=0.05 level. No explanation could be offered for the apparent antifertility observation at this plant, even though it was stated that EDB exposure for the entire group was <5 ppm.

Additional studies were undertaken to examine more directly the possible association between EDB exposure and antispermatogenic effects. The U.S. EPA examined 50 men who were grouped by occupation as follows: (1) citrus fumigators at two locations in Florida; (2) rice fumigators in Texas; (3) citrus fumigators in Texas; and (4) a mixed cohort of carrot farmers and researchers in New Jersey (Griffith et al., 1978). Sperm counts and measurements of serum testosterone, follicle stimulating hormone and luteinizing hormone were obtained for 44 of the men. Reliable measures of EDB exposure were not available, although a surrogate index (lifetime days of use times average pounds/day of use) was used to rank the potential severity of exposures. The results of the study indicated that sperm counts among the cohort members were within the normal range. A weak but statistically significant negative correlation was obtained between sperm count and lifetime days of EDB usage for the combined occupational groups (Spearman rank correlation: $r_s = -0.399$; $p<0.01$). There was no significant correlation between sperm count and average pounds/ day of EDB usage. These results suggest a possible relationship between prolonged exposure to EDB and sperm production. On the other hand, none of the participants reported difficulty in fathering children, and examination of sperm morphology in the 14 Florida citrus fumigators revealed no abnormalities.

Data were submitted to the U.S. EPA concerning a sperm count survey of 59 employees of an Ethyl Corporation EDB manufacturing plant at Magnolia, Arkansas in 1977 (Ter Haar, 1978, 1981). Concentrations of EDB in the air of the plant were reported to have ranged from nondetectable to 4.5 ppm, as an 8-hour TWA (Ter Haar, 1981). A total of 107 employees was considered eligible for inclusion in the study but 47 chose not to participate, and one failed to submit a semen sample. Analysis of individual sperm counts, as well as group evaluation based on cumulative EDB exposure and on job classification showed no deviations from normal. A subsequent sperm count performed in 1978 on 24 workers (including 18 from the previous year) again gave normal results (Ter Haar, 1981).

The study of the employees at the Ethyl Corporation plant in Magnolia, Arkansas, was extended in 1981 to evaluate possible adverse effects of EDB on fertility (Levine, 1981). Among the 167 persons employed at the plant, 164 were interviewed to determine their reproductive experience. Six female employees, ten male employees who had never been married, and 12 male employees with previous chemical exposures at other plants were subsequently excluded from the study. From the results of the questionnaire completed, no patterns of impaired fertility were evident. Fertility was reduced (non-significantly) among employees with <3 years of exposure; however, the reduction apparently did not correlate with estimated intensity of exposure. No statistically significant increases in numbers of miscarriages, stillbirths, neonatal deaths or birth defects were evident from the responses at the interview.

High Risk Subpopulations

The available epidemiologic evidence has not documented adverse effects in any human subpopulations. However, based on the results of animal studies (see Chapter V on Reproductive Effects and Chapter VIII on Quantification of Carcinogenic Effects), all human males can be considered at high risk of damage to the process of spermatogenesis. In addition, exposure to EDB in conjunction with inhibitors of drug metabolizing enzymes (e.g., disulfiram, diethyldithiocarbamate; see Chapter VII, Chapter VIII on Quantification of Carcinogenic Effects and Chapter V on Reproductive Effects) may constitute a high risk situation for persons of either sex.

Summary

The available data from case history reports indicate that EDB may be lethal to humans after a single oral dose of <65 mg/kg (Olmstead, 1960) and that severe local and systemic reactions can result from direct dermal contact (Pflesser, 1938). Mortality studies conducted on workers exposed to EDB are inconclusive with respect to cancer risk or death by other specific target organ effects (Ott et al., 1980; Turner, 1976, 1977). Because of limitations of scope and design, these epidemiologic studies are not considered to provide definitive results. In particular, small cohort size and lack of, or poorly characterized, exposure estimates are serious drawbacks. Several morbidity studies on EDB-exposed workers, only one of which is published, have focused on the evaluation of adverse effects on fertility (Griffith et al., 1978; Ter Haar, 1978, 1981; Wong et al., 1979). Again, the results are equivocal, showing only a slight indication of reduced fertility in two studies (Griffith et al., 1978; Wong et al., 1979), but no indication of reduced fertility (Levine, 1981) or impaired spermatogenesis (Ter Haar, 1978, 1981) in other studies. It must be concluded that the available epidemiologic evidence is not adequate to establish or deny that EDB affects human reproductive function, or is associated with the development of neoplastic or non-neoplastic chronic disease.

VII. MECHANISMS OF TOXICITY

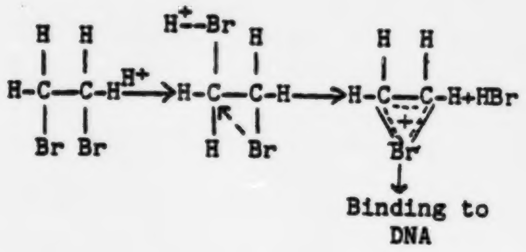
The mechanism of EDB toxicity is related intimately to its pattern of metabolism (see Chapter III). Binding to cell macromolecules may be considered the underlying cause of all EDB-induced genotypic and phenotypic effects, although this has not been established firmly. At least three mechanisms exist to explain the binding of EDB to cell macromolecules. EDB itself is a neutral molecule but can become in the cytosol an electrophile that possesses sufficient alkylating ability to bind directly with tissue constituents (van Bladeren et al., 1982). In addition to direct action, two reactive electrophilic intermediates may be formed during EDB metabolism: bromoacetaldehyde and a glutathione half-mustard, such as DNA, RNA and protein (Figure VII-1) (Livesey and Anders, 1979; van Bladeren et al., 1980, 1981a, 1982; Elliott and Ashby, 1980; Shih and Hill, 1981. The former metabolite arises from the cytochrome P-450-mediated microsomal oxidation of EDB, whereas the latter metabolite is an intermediate product of glutathione conjugation (see Figure VII-1). Both the oxidative and the conjugative pathways are operative in the rat, with the former pathway being predominant (van Bladeren et al., 1981a).

Current thinking, based on the role of glutathione conjugation in the mutagenicity of EDB, indicates that the conjugative pathway of metabolism results in bioactivation of EDB to an ultimate mutagen/carcinogen (Elliot and Ashby, 1980; van Bladeren et al., 1980, 1981a, 1982). This conclusion is supported by evidence of binding to DNA by a specific glutathione conjugate of EDB (Ozawa and Guengerich, 1983) and studies on the modifying influence of disulfiram on EDB mutagenicity (Elliot and Ashby, 1980; van Bladeren et al., 1981a, 1982). Evidence for the involvement of bromoacetal-

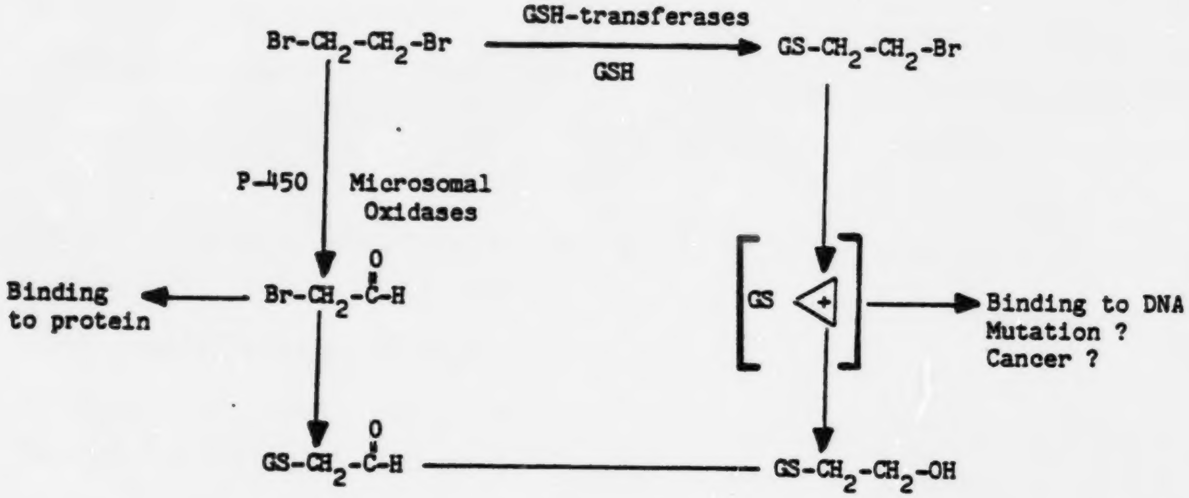
FIGURE VII-1

Involvement of Reactive Intermediates of EDB

Putative Nonenzymatic Electrophile Formation:


H H
H-C—C-H
Br Br
H+
H+--Br
H
H-C—C-H
H Br
H H
H-C—C-H+HBr
+
Br
Binding to DNA

Enzymatic Reactions:


Br-CH2-CH2-Br
GSH-transferases
GSH
GS-CH2-CH2-Br
P-450
Microsomal Oxidases
Binding to protein
Br-CH2-C(O)-H
GS
+
Binding to DNA
Mutation ?
Cancer ?
GS-CH2-C(O)-H
GS-CH2-CH2-OH

P-450 = cytochrome P-450

GSH = glutathione, reduced

dehyde in the production of toxic effects by EDB comes from studies that indicate a preferential binding of the aldehyde metabolite to microsomal proteins (Hill et al., 1978; Nachtomi, 1981; Shih and Hill, 1981). This type of protein binding would be expected to produce target organ tissue damage (i.e., necrosis) rather than mutation and cell transformation, although the latter cannot be ruled out.

The mechanism of action for the antispermatogenic effect of EDB in bulls and rams has not been established. At least one study suggests that the effect on the reproductive system results from the action of a metabolite rather than from EDB itself (Amir, 1973).

Synergistic Effects

The synergistic effect on carcinogenicity in rats of disulfiram with EDB has been well-documented (Wong et al., 1982). The enhancing effect of disulfiram on tumor production apparently is related to its ability to modify EDB metabolism. Disulfiram is an inhibitor of the enzyme aldehyde dehydrogenase, as well as cytochrome P-450-mediated oxidation reactions (van Bladeren et al., 1981a). Previous studies have indicated that disulfiram acts as an inhibitor of chemical carcinogenesis, thus, raising a paradox with EDB. Disulfiram when administered orally to rats causes a substantial decrease in excretion of urinary mercapturic acids derived from EDB (Plotnick et al., 1979; van Bladeren et al., 1981a). Since the major pathway of mercapturic acid formation from EDB in the rat is by oxidative metabolism followed by glutathione conjugation (van Bladeren et al., 1981a), it was concluded that bromoacetaldehyde production was not involved in the disulfiram-induced enhancement of EDB carcinogenicity. Rather, the formation of S-(2-bromoethyl)glutathione (and the resulting glutathione half-mustard) by direct conjugation of EDB with glutathione was implicated as the mechanism of bioactivation and further that disulfiram increased carcinogenicity by diverting metabolism of EDB from the oxidative pathway into the conjugative pathway (van Bladeren et al., 1981a). Supporting evidence was provided which showed that other inhibitors of cytochrome P-450 reactions (e.g., 1-phenylimidazole) produced similar reductions in mercapturic acid excretion in EDB-treated rats.

In addition to a synergistic effect on carcinogenicity, disulfiram also increased EDB-induced testicular atrophy in rats (Wong et al., 1982; see Carcinogenicity Section in Chapter V). Surprisingly, disulfiram had no effect on the production of testicular tumors in rats exposed to EDB, even though tumor incidence was increased dramatically in other organs.

Nachtomi (1980, 1981) determined that another enzyme inhibitor, diethyldithiocarbamate, also enhances the toxicity of EDB administered orally to rats. This compound is an inhibitor of cytochrome P-450-mediated reactions and was shown previously to be a hepatocellular protective agent against carbon tetrachloride and other carcinogens. In conjunction with EDB, diethyldithiocarbamate potentiated liver cell damage and incorporation of [methyl-^{3}H] thymidine into liver DNA _in_ _vivo_ while also decreasing the covalent binding of EDB to microsomal protein _in_ _vitro_. Pretreatment of rats with the microsomal enzyme inducer phenobarbital prevented the liver cell damage and effects on DNA synthesis induced by EDB. These results are consistent with a mechanism of synergistic action that involves enhancement of conjugative metabolism versus oxidative metabolism as was postulated also for disulfiram.

Summary

Although EDB is itself an electrophile and an alkylating agent, toxic effects resulting from its administration are attributed generally to the action of a reactive metabolite. Two intermediates are proposed as being principally responsible for the effects of EDB: bromoacetaldehyde and a glutathione half-mustard (see Figure VII-1) (Livesey and Anders, 1979; van Bladeren, 1980, 1981a, 1982; Shih and Hill, 1981). Microsomal oxidation reactions give rise to bromoacetaldehyde, which binds to microsomal proteins and presumably initiates tissue necrosis. Conjugation of EDB with glutathione and subsequent formation of a putative half-sulfur mustard is more closely associated with DNA binding and hence mutagenesis and carcinogensis (van Bladeren et al., 1980, 1981a, 1982; Ozawa and Guengerich, 1983). Bioactivation of EDB by glutathione conjugation is of considerable toxicologic significance simply because such conjugation reactions generally are believed to represent strictly a detoxication mechanism (Chasseaud, 1979).

The toxic action of EDB can be potentiated by pretreatment with, or concurrent administration of, certain enzyme inhibitors such as disulfiram and diethyldithiocarbamate (Nachtomi, 1980, 1981; van Bladeran et al., 1981a). Enhanced toxicity is evidenced by increased carcinogenic potency for EDB and also increased incidence of testicular atrophy in rats (Wong et al., 1982). This apparent synergism is believed to be caused by modified EDB metabolism, which results in the reduced formation of oxidative metabolites and increased production of glutathione conjugates (van Bladeren et al., 1981a,b).

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the noncarcinogenic and carcinogenic effects. In the quantification of noncarcinogenic effects, an Acceptable Daily Intake (ADI) is calculated. An Adjusted Acceptable Daily Intake (AADI) and Health Advisory (HA) values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{\text{(NOAEL or LOAEL in mg/kg/day) (body weight in kg)}}{\text{uncertainty/safety factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking Water Volume in } \ell\text{/day}} = \text{mg/}\ell$$

where:

NOAEL = no-observed-adverse-effect level

LOAEL = lowest-observed-adverse-effect level

Body weight = 70 kg for adult or 10 kg for child

Drinking water volume = 2 ℓ/day for adults or 1 ℓ/day for children

Uncertainty/Safety Factor = 10, 100 or 1000

Utilizing these equations, the following drinking water concentrations are developed for noncarcinogenic effects:

1. A 1-day HA for 10 kg child.
2. A 1-day HA for 70 kg adult.
3. A 10-day HA for 10 kg adult.
4. A 10-day HA for 70 kg adult.
5. A lifetime AADI for a 70 kg adult.

The distinctions made between the HA calculations (items 1 through 4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1 to 2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon noncarcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivity to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS, 1977, 1980) are typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data are available and supported by acute or chronic toxicity data in other species.
- An uncertainty factor of 100 is used when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more species, but human data are not available.

- An uncertainty factor of 1000 is used when limited or incomplete acute or chronic toxicity data identifying a LOAEL (but not NOEL/NOAEL) for one or more species are available.

The uncertainty factor used for a specific risk assessment is judgemental. Factors that cannot be incorporated in the NAS/ODW guidelines for selection of an uncertainty factor, but must be considered include the following: (1) the quality of the toxicology data, (2) the significance of the adverse effect and (3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen, mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical through drinking water. There is adequate evidence to classify EDB as an animal carcinogen following oral exposure. The bioassay data used in these estimates are from animal experiments. In order to predict the risk for humans, these data must be converted to an equivalent human dose. This conversion includes correction for noncontinuous animal feeding, nonlifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 ℓ water/day. The multi-stage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as (1) incomplete knowledge concerning the health effects of contaminants in drinking water, (2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results and (3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Current Regulations and Standards

The U.S. EPA (Federal Register, 1983a) has recently suspended the use of the pesticide EDB as a soil fumigant and is limiting the use of this substance in fumigation of citrus fruits. The tolerances for EDB on food products have been set at 900 ppb for raw grain for human consumption, 150 ppb for flour and 30 ppb for ready-to-eat products.

Occupational exposure limits to EDB have also been recently lowered. The Occupational Safety and Health Administration (OSHA) (Federal Register, 1983b) has lowered the 8-hour TWA exposure from 20 ppm to 0.1 ppm in workroom air. The new short-term exposure is 0.5 ppm over 15 minutes. The

American Conference of Governmental Industrial Hygienists (ACGIH, 1984) classifies EDB as A2, which indicates that the compound is suspected of carcinogenic potential for man. The ACGIH (1984) states that all exposure should be carefully controlled, but does not provide a suggested TWA limit.

Noncarcinogenic Effects

Limited data are available regarding the acute effects of orally administered EDB. Single dose oral LD_{50}s of 117-146, 420, 55 and 100 mg/kg have been reported for rats, mice, rabbits and guinea pigs, respectively (Rowe, et al., 1952). Single oral doses of 75-120 mg/kg have produced evidence of hepatotoxicity (e.g., hepatomegaly, centrilobular necrosis, increased levels of liver lipids and serum enzymes, evidence of DNA damage and repair) in rats (Nachtomi, et al., 1968; Nachtomi and Alumot, 1972; Broda, et al., 1976; Nachtomi and Farber, 1978; Nachtomi and Sarma, 1977; Nachtomi, 1980). Administration of EDB by gavage at a dose of 10 mg/kg bw/day for 12 days produced elevated levels of serum GPT and SDH in rats, but dietary administration at a reported level of ~10-20 mg/kg/day for 18 days did not result in a significant increase in liver weight, DNA content or thymidine incorporation (Nachtomi, 1980). Symptoms of toxicity and effects at sites other than the liver were not described in any of the aforementioned acute oral studies with rodents, but it was reported in a study with three dogs that CNS depression was a primary effect of lethal single gavage doses ($\geq$62 mg/kg) (Kistler and Luckhardt, 1929). A clinical case report indicated that EDB may be lethal to humans after a single oral dose of >65 mg/kg (Olmstead, 1960).

Maximum nonfatal exposures for rats exposed to EDB vapor ranged from 1.2 minutes at 10,000 ppm to 16 hours at 100 ppm (Rowe et al., 1952). Guinea pigs survived exposure to 200 ppm EDB for 7 hours and 400 ppm for 2 hours, but not to 400 ppm for longer durations (≥3 hours). Deaths generally occurred within 24 hours at the higher concentrations and were caused by respiratory or cardiac failure. Deaths at lower concentrations were usually caused by pneumonia resulting from lung injury and typically were delayed. Pathological effects in rats that were subjected to lethal single EDB exposure were found in the lungs (e.g., congestion, edema, hemorrhages, inflammation), liver (e.g., cloudy swelling, centrilobular fatty degeneration and necrosis) and kidney (e.g., congestion, edema and cloudy swelling). Additional experiments with groups of 10 female rats indicated that exposure to 100, 200 or 800 ppm produced adverse or no adverse effects in the liver (increased weight and "slight" histopathological changes were considered to be the adverse effects) after 4.0 or 2.5 hours, 1.0 or 0.7 hours and 0.15 or 0.10 hours, respectively (Rowe et al., 1952); 50 ppm for 7.0 hours reportedly did not produce adverse liver effects as indicated by these criteria, but an exposure that elicited adverse liver effects at 50 ppm was not determined. Exposure of groups of 23 rats or 8 guinea pigs to 25 ppm EDB for 13 seven-hour expcsures in 16-17 days did not produce adverse effects as judged by behavior, growth, body or organ weights, BUN levels, hematologic indices (rats), total liver lipid content (rats), tissue histology or mortality, but similar exposures to 50 ppm produced increased liver and kidney weights in rats and depressed weight gain in guinea pigs (Rowe et al., 1952). Rats and rabbits exposed 7 hours/ day for 4-9 days to 100 ppm EDB showed some mortality and histopathologic effects (e.g., cloudy swelling or necrosis in the liver, thickened alveolar walls in rats, slight splenic congestion and hemosiderosis in rats (Rowe et al., 1952).

Application of EDB to the skin of small numbers of rats at doses of ~1.5-6 mg/kg bw (Thomas and Yant, 1927) or rabbits at 0.2-1.1 mg/kg bw (Rowe et al., 1952) produced local (erythema, edema) and systemic (CNS depression) toxic effects and mortality. Severe local and systemic reactions have been produced in human volunteers by direct dermal application of EDB (Pflesser, 1938).

Range-finding studies for a carcinogenesis bioassay were conducted in which rats and mice were treated by gavage with doses of 0, 40, 63, 100, 159-163 or 251 mg/kg EDB 5 days/week for 6 weeks (NCI, 1978); the corresponding TWA doses were 0, 28.6, 45, 71.4, 113.6-116.4 or 179.3 mg/kg/day, respectively, when extrapolation to a 7-day/week exposure regimen is made. Only two criteria were evaluated (body weight and mortality); neither parameter was altered significantly in rats at $\leq$45 mg/kg/day or in mice at 28.6 mg/kg/day.

Data on the non-neoplastic effects of chronic oral exposure to EDB are available from the NCI (1978) carcinogenesis bioassay in which Osborne-Mendel rats and B6C3F$_1$ mice of both sexes were exposed by gavage to TWA doses of 0, ~27 or 28-29 mg/kg/day for 49-61 weeks and 0, ~44 or 77 mg/kg/day for 53 weeks, respectively. It should be noted that the high-dose rat groups initially were exposed at a dose of ~57 mg/kg/day, but that early mortality prompted discontinuation of treatment during weeks 17-29 as detailed in the Carcinogenicity Section of Chapter V. Significant mortality that may have been tumor-related (forestomach carcinoma) occurred in both low- and high-dose rats and mice. Treatment-related non-neoplastic effects were found in the forestomach (hyperkeratosis and acanthosis in the high-dose male rats, male and female mice, and in the low- and high-dose female rats), liver (peliosis hepatitis or inflammation in the low- and high-dose male and female rats), adrenal cortex (degeneration in the low- and high-dose male rats and high-dose female rats) and testes (atrophy in the low- and high-dose rats and high-dose mice).

Inhalation exposure to EDB at a concentration of 25 ppm for 145-156 seven-hour exposures in 205-220 days did not produce adverse effects in groups of 40 rats, 16 guinea pigs, 4 rabbits or 2 monkeys as determined by behavior, growth, body or organ weights, BUN levels, hematological indices (rats, monkeys), total liver lipid (monkeys) or tissue histology, but mortality from respiratory infection resulting from lung damage occurred in 13/40 rats and 6/8 guinea pigs (Rowe et al., 1952). In other subchronic inhalation studies, F344 rats (4-6 per dose group) were exposed to 0, 3, 15 or 75 ppm EDB vapor 6 hours/day, 5 days/week for 13 weeks (NTP, 1982). Dose-related depressed body weight gain occurred in the male rats (8% depression at 3 ppm), male mice (21% depression at 15 ppm) and female mice (15% depression at 3 ppm). Mortality occurred in 4/10 male mice exposed at 3 ppm, but in none of the higher dose mice except 1/20 females at 75 ppm; no mortality occurred in the rats. Histopathological effects occurred only at 75 ppm (e.g., nasal cavity hyperplasia or squamous metaplasia in rats and mice, adrenal cortical cell swelling/vacuolization in rats, decreased thyroid size in rats). In another study, slight and reversible epithelial hyperplasia of the nasal cavity was noted in F344 rats that were exposed to 10 ppm EDB (6 hours/day, 4 days/week for 13 weeks), but consistent histological effects were not apparent at 3 ppm (Nitschke, 1980).

Chronic inhalation exposure to EDB at concentrations of 1, 10 or 40 ppm (6 hours/day, 5 days/week for 78-103 weeks) produced increased mortality in male and female F344 rats at the high dose and increased mortality in female B6C3F$_1$ mice at the low and high doses (31/50 and 43/50, respectively, vs. 10/50 in controls) (NTP, 1982). Treatment-related non-neoplastic lesions occurred in the respiratory system (epithelial hyperplasia, squamous metaplasia or inflammation of the nasal cavity, bronchus or lung) in the low- and high-dose rats and mice of both sexes, liver (necrosis) in the high-dose rats of both sexes, kidneys (toxic nephropathy) in low- and high-dose male rats and high-dose female rats, testis (degeneration and atrophy) in low- and high-dose rats, and adrenal cortex (degeneration) and retina (atrophy) of low- and high-dose female rats. In another chronic inhalation study, mortality was increased and the incidence of atrophy of the spleen was elevated significantly (6/48 vs. 0/48 in controls) in groups of 48 Sprague-Dawley rats that were exposed to 20 ppm EDB (7 hours/day, 4 days/week for 18 months), but testicular atrophy was not found and the nasal cavity was not examined (Wong et al., 1982).

Mortality studies that were conducted on workers who were subchronically/chronically exposed to EDB are inconclusive with respect to cancer risk or death by other specific target organ effects (Turner, 1976, 1977; Ott et al., 1980). Limitations including small cohort sizes, poorly characterized exposure levels and concomitant exposure to other chemicals indicate that these results should not be regarded as definitive.

Exposure of pregnant CD rats or CD-1 mice to 20 ppm EDB nearly continuously (23 hours/day) on days 6-15 of gestation produced reduced maternal feed consumption with (mice) and without (rats) significant weight loss, increased percentage of late resorptions and decreased fetal weight in the mice, reduced frequency of normally ossified centra in the rats (an effect that was not seen at higher exposure levels and that occurred with increased frequency in a diet-restricted unexposed control group), and incompletely ossified supraoccipital and unossified incus in the mice. Similar exposure to 32 ppm produced hydrocephaly, wavy ribs, a lower frequency of the 14th pair of ribs in rats and an increased incidence of skeletal ossification anomalies in mice (Short et al., 1976). There was significantly reduced feed consumption and weight loss in the maternal rats and mice, and only two of the ossification effects in the mice (incompletely ossified/unossified sternebrae) occurred at incidences that were significantly higher than in feed-restricted controls. Reduced maternal feed consumption and body weight and fetotoxicity (reduced fetal weight) were observed in rats similarly exposed to 38 ppm EDB, but fetal developmental effects were not noted (Short et al., 1978). Similar exposure to 38 ppm produced maternal mortality, marked embryotoxicity, a high percentage of runts and numerous fetal ossification anomalies in mice, but diet-restricted unexposed control data were not available. Gestational exposure to 80 ppm EDB produced 50% maternal mortality and pronounced fetolethality in rats, and 100% maternal mortality in mice (Short et al., 1978). The results of the above studies show that the fetal anomalies occurred at doses that were maternal/fetotoxic and were not dose-related, and indicate that the effects should be regarded as a manifestation of fetal toxicity (i.e., delayed development) rather than teratogenic action. Intraperitoneal injection of 55 mg/kg EDB to Sprague-Dawley rats produced unspecified alterations in the weights of two unspecified organs in maternal rats, but no evidence of fetotoxicity or developmental anomalies (Hardin et al., 1981); the results of this study were not reported in detail.

Dietary administration of EDB to bulls at an average daily dose of 2 mg/kg, beginning at 4 days of age to 14-16 months of age or from 14-16 months of age, produced reversible antispermatogenic effects without other evidence of toxicity as detailed in the Reproductive Effects Section of Chapter V (Amir and Volcani, 1965). Antispermatogenic effects were evident as early as 2 weeks after initiation of treatment. A high percentage of abnormal spermatozoa also was produced in bulls after 10 oral doses of 4 mg/kg EDB given on alternate days (Amir and Ben-David, 1973; Amir and Lavon, 1976), or given as a single peritesticular injection of 110-120 mg (Amir and Ben-David, 1973) or 270 mg/kg (Amir et al., 1979). The reversible antispermatogenic effect of EDB seen in bulls also has been produced in rams following 12 consecutive daily subcutaneous injections of 7.8-13.5 mg/kg (acute systemic toxicity was evident at 16.9 mg/kg/day) (ElJack and Hrudka, 1979) and by single peritesticular injections of 250 or 430 mg/kg (Amir et al., 1983) but not by chronic oral administration (dose not reported) (Amir and Ben-David, 1973). Rats may be relatively resistant to the spermicidal action of EDB since adverse effects on fertility were produced by inhalation only at levels of exposure (89 ppm for 7 hours/day, 5 days/week for 10 weeks) associated with systemic toxicity (Short et al., 1979). Testicular atrophy, however, has been demonstrated in rats and mice that were exposed chronically to EDB by gavage or inhalation as detailed earlier (NCI, 1978; NTP, 1982; Wong et al., 1982). A slight indication of reduced fertility was reported in two human morbidity studies (Griffith et al., 1978; Wong et al., 1979), but indication of reduced fertility (Levine, 1981) or impaired spermatogenesis (Ter Haar, 1978, 1981) were not found in other studies. The results of these studies must be regarded as equivocal due to limitations of scope and design.

Quantification of Noncarcinogenic Effects

1-Day Health Advisory. The most appropriate data available for the derivation of short-term health advisories are from the ElJack and Hrudka (1979) study in sheep. Since the exposure duration in this study was 12 days, these data will be used to calculate the 10-day HA directly. It is recommended that the 10-day HA be used as a 1-day HA (see below for calculations).

10-Day Health Advisory. The study by ElJack and Hrudka (1979) has been chosen to serve as the basis for the 10-day HA. In this study, 18 rams were given EDB at 7.8, 9.6 or 13.3 mg/kg/day subcutaneously for 12 consecutive days. Substantial effects on the testis were noted. These effects were included reduction in motility and an increase in the number of morphologically abnormal and degenerating sperm. The severity of the response was dose-dependent. A NOAEL could not be identified in the study, but the lowest dose can be considered a LOAEL, since the changes observed in the parameters measured ranged from 10-15% below control levels.

The 10-day HA is calculated as follows:

For the 10 kg child:

$$\frac{(7.8\ \text{mg/kg/day})\ (10\ \text{kg})}{(100)\ (10)\ (10)\ (1\ \ell/\text{day})} = 8.0\ \mu\text{g}/\ell$$

where:

7.8 mg/kg/day = LOAEL in rams for reproductive effects

10 kg = assumed weight of protected individual

100 = uncertainty factor, appropriate for use in inter- and intraspecies extrapolation

10 = uncertainty factor, appropriate for use with LOAEL when NOAEL has not been identified

10 = uncertainty factor, considered appropriate to accommodate for possibility that the human is closer in sensitivity to the bull than to the ram*

1 ℓ/day = assumed volume of water ingested per day by 10 kg child in liters

For the 70 kg adult:

$$\frac{(7.8\ \text{mg/kg/day}\ (70\ \text{kg})}{(100)\ (10)\ (10)\ (2\ \ell/\text{day})} = 28.0\ \mu\text{g}/\ell$$

where:

70 kg = weight of protected individual

2 ℓ/day = assumed volume of water consumed/day for a 70 kg adult (Other factors as described above for 10 kg child)

Lifetime Adjusted Acceptable Daily Intake (AADI). The most appropriate data for derivation of a lifetime AADI would be results obtained from chronic oral studies/bioassays using a suitable mammalian species, since equivalent human data do not exist. Several chronic and subchronic studies have been conducted in a number of species, principally rodents, exposed orally to EDB. Most of these studies identify dose-response relationships for a variety of noncarcinogenic endpoints. However, none of them adequately define NOAELs.

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*A series of studies in bulls has been published in which a total of 24 animals were treated orally for periods ranging from 20 days to approximately 2 years (see Reproductive/Teratogenic Effects Section). Only one dose level (4 mg/kg every other day) was given in these experiments. While the data from these studies are not considered suitable for quantitative risk assessment, they do show that the bull is more sensitive to the effects of EDB than is the ram. Since no evidence exists to conclude that the human male is closer in sensitivity to the bull, to the ram, or even to the rodent species, an added uncertainty factor of 10 has been utilized to account for the possibility that the human is as sensitive as the bull.

Groups of 50 male and female B6C3F$_1$ mice received TWA doses of 43.6 or 43.9 mg/kg/day, respectively, of EDB by gavage for 53 weeks (NCI, 1978). In both sexes, body weight gain was depressed. Forestomach hyperkeratosis was seen in 1/49 females; these effects were not seen in the controls. These exposure levels could be considered a LOAEL for the mouse.

In similar studies in rats, on the other hand, the apparent LOAEL is smaller than for the mouse. Groups of Osborne-Mendel rats received EDB by gavage at average doses of 27.4 and 29.1 mg/kg/day for 49 weeks (males) or 26.7 or 28.1 mg/kg/day for 61 weeks (females) (NCI, 1978). At the lower level of exposure in males, testicular atrophy (14/49) and liver degeneration were seen, neither of which occurred in the controls. In the females at the lower dose, body weight gain was depressed and forestomach hyperkeratosis (4/50), forestomach acanthosis (4/50) and liver inflammation (4/47) were seen; none of these observations were made in the controls. These effects are rather severe; it is entirely possible that lower observed adverse effect levels would be found for ingested EDB in the rat if tested.

Furthermore, long-term exposure data for EDB in the bull indicate that an oral NOAEL or LOAEL for nonrodents may be considerably lower than the doses tested in the rodents. These data have been described in detail above for the bull and ram and would lead to the conclusion that an oral NOAEL would be below 2 mg/kg/day and that lower observed adverse effect levels are possible. In rodents, testicular effects were observed at dose levels that also exerted other toxic effects. In the bull, in particular, the anti-spermatogenic effects occurred in the absence of adverse effects as measured by growth, general health or libido.

Several long-term studies have been conducted during which rodents have been exposed to EDB vapors. Results from these studies may be compared with data from the oral studies to establish equivalency of absorbed dose and toxic effects. At the lowest concentration tested in male F344 rats (10 ppm for 6 hours/day, 5 days/week for 103 weeks), EDB produced testicular degeneration (10/50 vs. 1/50 in the controls) (NTP, 1982). This level of exposure would correspond to a total absorbed dose of EDB of ~4.6 mg/kg/day, assuming a daily inhalation volume of 0.223 m^3, an inhalation absorption efficiency of 50% and a body weight for the rat of 0.35 kg (i.e., 76.8 mg/m^3/day x 0.233 m^3/day x 6/24 hours x 5/7 days x 0.5 divided by 0.35 kg). The lowest concentration of EDB tested in Sprague-Dawley rats by chronic inhalation was 20 ppm; this concentration caused frank adverse effects (mortality and spleen atrophy) (Wong et al., 1982).

Male and female B6C3F$_1$ mice (50 per group) have been exposed to EDB vapors at concentrations as low as 10 ppm (6 hours/day, 5 days/ week) for periods of 78 weeks (males) or 103 weeks (females) (NTP, 1982). This concentration appears to be a LOAEL in the mouse for non-neoplastic effects. This level of exposure corresponds to a daily absorbed dose of EDB of ~9.1 mg/kg/day, assuming a daily inhalation volume of 0.039 m^3/day and a body weight of 0.03 kg for the mouse and an inhalation absorption efficiency of 50% (78.6 mg/m^3 x 0.039 m^3/day x 6 hours/24 hours x 5 days/7 days x 0.5 ÷ 0.03 kg).

It is evident from the above discussion that a LOAEL can only be identified for the mouse exposed by inhalation, while frank effects were observed in rats exposed to the same concentration (10 ppm). Estimated absorbed doses corresponding to the 10 ppm exposure in these species would be in the range of 4-9 mg/kg/day, levels that are slightly higher, but consistent with the daily oral intakes that have been reported to produce reproductive toxicity in bulls.

No one nonprimate animal model has been identified as being the best surrogate for the human when studying reproductive effects, either in the male or the female. Thus, to remain in accordance with the general philosophy that data used in the extrapolation from animal studies to the human be drawn from the most sensitive species, it is appropriate, in this case, to use the antispermatogenic effects of EDB in bulls as the point of reference. From the data available on the bull, however, it is not possible to accurately identify a NOAEL for noncarcinogenic endpoints of toxicity following long-term exposures by any route of exposure; sufficient data are not available to place confidence in the LOAELs. Thus, it is not possible to estimate with any accuracy an AADI for EDB at this time.

Carcinogenic Effects

EDB has been demonstrated to be a potent carcinogen in rats and mice exposed by gavage and inhalation. When administered by gavage to Osborne-

Mendel rats at TWA doses of ~27-29 mg/kg/day for 49 or 61 weeks, EDB produced significantly increased incidences of squamous cell carcinomas of the forestomach of both sexes, hemangiosarcomas of the circulatory system in males and hepatocellular carcinomas and liver neoplastic nodules (NCI, 1978) (see Carcinogenicity Section of Chapter V). Similar administration to $B6C3F_1$ mice at average doses of ~44 or 77 mg/kg/day for 53 weeks induced squamous cell carcinomas of the forestomach and alveolar/bronchiolar adenomas in both sexes. Forestomach carcinomas developed in 55-90% of the treated rats and mice (none were found in vehicle controls); occurrence of these tumors was dose-related.

Inhalation exposure to EDB at concentrations of 10 or 40 ppm, 6 hours/day, 5 days/week for 78-103 weeks produced significantly increased tumor incidences of nasal cavity tumors (particularly adenocarcinomas, carcinomas and adenomatous polyps) in F344 rats of both sexes (NTP, 1982), alveolar/bronchiolar carcinomas or adenomas in female F344 and $B6C3F_1$ mice of both sexes (NTP, 1982) and nasal cavity tumors (particularly carcinomas) in female $B6C3F_1$ mice (Stinson et al., 1981; NTP, 1982). Significantly increased incidences of circulatory system hemangiosarcomas (male and female rats, female mice), pituitary adenomas (male and female rats), tunica vaginalis mesotheliomas (female mice) and subcutaneous fibrosarcomas (female mice) also were found in the NTP (1982) inhalation study. The results of another chronic inhalation study in which Sprague-Dawley rats were exposed to 20 ppm EDB for 7 hours/day, 5 days/week for 72 weeks are consistent with those of the NTP (1982) bioassay (Wong et al., 1982). In this study with Sprague-Dawley rats, significantly increased incidences of hemangiosarcomas (males and females), adrenal tumors (males and females), subcutaneous mesenchymal tumors (males) and mammary gland tumors (females) were induced; the nasal cavity was not, however, examined in this study.

In a lifetime skin application study with female ICR/Ha Swiss mice, thrice weekly application of 25 or 50 mg EDB (~830 or 1670 mg/kg bw, respectively) to the dorsal skin caused significantly increased incidences of local skin papillomas and/or lung papillomas, but no carcinomas (Van Duuren et al., 1979). Initiation with a single dermal dose of 75 mg EDB followed 14 days later by thrice weekly promotion with 2.5 or 5 μg phorbol myristate acetate (PMA) for life did not, however, produce a significant increase in the incidence of local papillomas or carcinomas in the same strain of mice.

The carcinogenic potential of EDB is supported further by positive evidence of mutagenicity. As detailed in the Mutagenicity Section of Chapter V, EDB has been demonstrated to be mutagenic _in vitro_ in bacteria and eukaryotic cells. _In vivo_ exposure of higher organisms (i.e., _Tradescantia_ and _Drosophila_) to EDB also has resulted in mutations, but EDB did not induce dominant lethal mutations in mice or rats. Limited evidence suggests that EDB may cause clastogenic effects such as sister chromatid exchanges, chromosomal aberrations and polyploidy. Interaction with DNA has been demonstrated by both indirect (preferential killing of DNA repair-deficient bacteria, induction of unscheduled DNA synthesis and single strand breaks in DNA) and direct (binding to DNA) methods. The major EDB adduct formed has been identified tentatively as S-[2-(N^7-guanyl)ethyl] glutathione.

The results of one published (Ott et al., 1980) and two unpublished (Turner, 1976, 1977) retrospective mortality studies do not indicate a significantly increased risk of death from cancer in EDB-exposed workers (see Epidemiological Studies Section in Chapter VI). The results of these studies should, however, be regarded as inconclusive because of the limitations of scope and design that include inadequate exposure monitoring data, concomitant exposure to other chemicals and/or small cohort size.

Quantification of Carcinogenic Effects

The clearest evidence of a potential cancer risk from ingestion of EDB is found in the NCI (1978) report. A high incidence of forestomach carcinoma was observed in all exposure-species-sex groups in a relatively short period of time.

To make use of this data a mathematical model that incorporated variable partial lifetime exposure was developed. The details of this model are explained in a paper by Thorslund (1982) presented at the Pacific Division AAAS meeting in Santa Barbara, California.

In that paper a human risk at age t due to an exposure of d mg/kg/day from age s to f was estimated from the following equation:

$$P(t,d) = 1-e^{-6.958 \times 10^{-14} \times d \times [(t-s)^{7.6}-(t-f)^{7.6}]}$$

However, that equation was based upon an equivalency assumption between species on a mg/kg basis. Assuming mg/surface area exposure equivalency, 70 kg human and a 500 g rat, the exponent in the above equation is multiplied by $(70/0.5)^{1/3} = 5.192$, which gives the following result:

$$P(t,d) = 1-e^{-3.613 \times 10^{-13} \times d \times [(t-s)^{7.6}-(t-f)^{7.6}]}$$

To estimate the exposure level of EDB in drinking water that will result in a specified risk P(t,d) we solve the above equation for d giving the following result:

$$d = -\ln[1-P(t,d)]/(3.613 \times 10^{-13} \times [(t-s)^{7.6} - (tpf)^{7.6}])$$

We calculate such exposure levels for a 10 kg child, age 1 year, assuming 1 ℓ/day drinking water consumption and adults age 20 or 55 consuming 2 ℓ/day drinking water. This is done for a 1-day, 10-day, or rest of lifetime duration of exposure for risk levels of 1×10^{-4}, 1×10^{-5}, 1×10^{-6}. These results are shown in Table VIII-1 where t is set equal to 76.2 (the median lifespan). The levels are shown for a risk of 1×10^{-4}. To equate these levels to a 1×10^{-5} or 1×10^{-6} risk, divide by 10 or 100, respectively.

Special Considerations

Synergistic Effects. The increased risk associated with concurrent exposure to EDB with certain enzyme inhibitors is well-documented (see Synergistic Effects Section of Chapter VII). In particular, the microsomal oxidase and aldehyde dehydrogenase inhibitor disulfiram enhances both the

TABLE VIII-1

ppm EDB in Drinking Water that Results in Cancer Risk of 1×10^{-4}

Duration of Exposure (f-s) in Years	Age at First Exposure s, in Years		
	s = 1	s = 20	s = 55
1 Day = 1/365 = 0.00274	5.51×10^{-2}	1.32×10^{0}	8.23×10^{2}
10 Days = 10/365 = 0.0274	5.51×10^{-3}	1.32×10^{-1}	8.23×10^{1}
Rest of lifetime (76.2-s)	5.02×10^{-5}[a]	4.88×10^{-4}	8.05×10^{-1}

[a] assumes that on average over lifespan age 1-76.2 years that 1 ppm in drinking water is equivalent to 33 μg/kg bw.

toxic and carcinogenic effects of EDB in rats (Wong et al., 1982). This effect has been attributed to an alteration in the pathway of EDB metabolism, which results in the increased formation of glutathione conjugates (Plotnick et al., 1979; Elliot and Ashby, 1980; van Bladeren et al., 1981a). Since the conjugative pathway of EDB metabolism is associated with the formation of a putative highly reactive glutathione half-mustard intermediate, enhanced toxicity apparently is due to increased binding to cell macromolecules (Banerjee and Van Duuren, 1979; van Bladeren, 1980, 1981a, 1982; Shih and Hill, 1981; Ozawa and Guengerich, 1983). The microsomal inhibitor diethyldithiocarbamate also potentiates the toxicity of EDB in the rat (Nachtomi, 1980, 1981), presumably acting by the same mechanism as disulfiram. Conversely, the microsomal enzyme inducer butylated hydroxytoluene affords protection against the acute toxicity of EDB to mice (Cumming and Walton, 1973), presumably by favoring the production of oxidative rather than conjugative metabolites.

High Risk Subpopulations. Because of the well-documented antispermatogenic effect of EDB in animals (see Reprodutive Effects Section in Chapter V), human males should be considered as a population at special risk. Presently, the available evidence concerning adverse reproductive effects in EDB-exposed workers is inconclusive. Nevertheless, in occupations involving direct contact with EDB (i.e., fumigators), special precautions should be taken to minimize exposure.

An additional risk with EDB involves situations where humans may be exposed simultaneously to inhibitors of drug metabolizing enzymes. Because disulfiram is used therapeutically in the medical management of alcoholism, these individuals may be at increased risk if exposed to EDB. Any substance that has the potential to inhibit the oxidative metabolism of EDB should be regarded as posing a special threat to those persons in simultaneous contact.

Summary

Although quantitative data are available in rodents from which HAs and AADIs possibly could be derived, it is concluded that the antispermatogenic effects observed in bulls and rams must be considered as the most relevant experimental data to the human. Having made that choice, it is concluded that both the 1-day and 10-day HA are 8 and 28 μg/ℓ for a 10 kg child or a 70 kg adult, respectively. An AADIs cannot be proposed, since no adequate dose-response data in the bull or ram exist from which this latter number could be derived.

EDB is an unequivocal carcinogen in rats and mice exposed by inhalation or gavage. Lifetime excess cancer risks for EDB in groundwater were estimated by the U.S. EPA Carcinogen Assessment Group on behalf of the Office of Pesticide Programs (U.S. EPA, 1983). Estimates were derived from tumor responses in male rats in the 1978 NCI gavage bioassay. These estimates are modified in this document to accommodate for a differing assumption on the weight of the protected individual, from 60 kg for OPP to 70 kg for ODW.

The documented antispermatogenic effect of EDB in mammals indicates that human males should be regarded as a population at special risk. An additional risk with EDB involves situations where humans may be exposed simultaneously to inhibitors of drug metabolizing enzymes (i.e., therapeutic use of disulfiram in the medical management of alcoholism).

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An additional risk with EDB involves situations where humans may be exposed simultaneously to inhibitors of drug metabolizing enzymes. Because disulfiram is used therapeutically in the medical management of alcoholism, these individuals may be at increased risk if exposed to EDB. Any substance that has the potential to inhibit the oxidative metabolism of EDB should be regarded as posing a special threat to those persons in simultaneous contact.

Summary

Although quantitative data are available in rodents from which HAs and AADIs possibly could be derived, it is concluded that the antispermatogenic effects observed in bulls and rams must be considered as the most relevant experimental data to the human. Having made that choice, it is concluded that no HAs or AADIs can be proposed, since no adequate dose-response data in the bull or ram exist from which these numbers could be derived.

EDB is an unequivocal carcinogen in rats and mice exposed by inhalation or gavage. Lifetime excess cancer risks for EDB in groundwater were estimated by the U.S. EPA Carcinogen Assessment Group on behalf of the Office of Pesticide Programs (U.S. EPA, 1983). Estimates were derived from tumor responses in male rats in the 1978 NCI gavage bioassay. These estimates are modified in this document to accommodate for a differing assumption on the weight of the protected individual, from 60 kg for OPP to 70 kg for ODW.

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